August 1991

YAJSJMA HATCHERY EXPERIMENTAL DESIGN

Annual Report 1991

DOE/BP-00102

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views of this report are the author's and do r necessarily represent the views of BPA.

This document should be cited as follows:

Busack,Craig; Curtis Knudsen, Anne Marshall, Stevan Phelps, Dave Seiler, Washington Department of Fisheries, Tom Clune, Project Manager, U. S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Project No. 1989-082, Contract Number DE-B179-89BP00102, (BPA Report DOE/BP-00102)243 electronic pages

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YAKIMA HATCHERY EXPERIMENTAL DESIGN

Annual Progress Report

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Project No. 89-082
Contract Number DE-B179-89BP00102

August, 1991

CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF APPENDICES

FOREWORD

This progress report details the results and status of Washington Department of Fisheries' (WDF) pre-facility monitoring, research, and evaluation efforts, through May 1991, designed to support the development of an Experimental Design Plan (EDP) for the Yakima/Klickitat Fisheries Project (YKFP), previously termed the Yakima/Klickitat Production Project (YKPP or Y/KPP). This pre-facility work has been guided by planning efforts of various research and quality control teams of the project that are annually captured as revisions to the experimental design and pre-facility work plans. The current objectives are as follows:

- Develop genetic monitoring and evaluation approach for the Y/KPP.
  - definegeneticallydistinctsub-populations of steelhead and chinook in the Yakima and Klickitat River basins.
  - assess genetic risk of various project options.
  - develop experimental design and monitoring and evaluation strategies to evaluate long-term genetic change and associated reproductive performance.
- Evaluate stock identification monitoring tools, approaches, and opportunities available to meet specific objectives of the experimental plan.
- Evaluate adult and juvenile enumeration and sampling/collection capabilities in the Y/KPP necessary to measure experimental response variables.

Because these objectives and related tasks represent a wide range of activities, we have presented our work progress in three respective, self-contained reports herein: REPORT NO. 1: POPULATION STRUCTURE AND GENETICS; REPORT NO. 2: STOCK IDENTIFICATION MONITORING TOOLS; and REPORT NO. 3: EVALUATION OF JUVENILE AND ADULT MONITORING. Various aspects of this work are on-going, at least through December 31, 1991, and future direction will be shaped by current YKFP planning efforts.

REPORT NO. 1

POPULATION STRUCTURE AND GENETICS

- Genetic Analysis of YKFP Chinook Salmon Stocks.
  by Craig Busack and Anne Marshall

- Genetic Analysis of Yakima River Steelhead: Initial analysis of within-basin genetic diversity and comparison to hatchery steelhead and rainbow trout.
  by Stevan R. Phelps

- YKFP Genetic Risk Assessment.
  by Craig Busack

- Genetic Monitoring Aspects of the YKFP.
  by Craig Busack

- Scale Pattern and Age/Length Analysis of 1989 and 1990 Yakima River Adult Spring Chinook.
  by Curtis M. Knudsen

- Yakima Steelhead and Rainbow Trout Age, Length, and Scale Pattern analyses.
  by Curtis M. Knudsen

GENETIC ANALYSES OF YKFP CHINOOK SALMON STOCKS

INTRODUCTION

The central hypothesis of the YKFP is that state-of-the-art supplementation procedures can be used to increase production of salmon and steelhead in the Yakima and Klickitat sub-basins without adversely affecting the genetic resources present. Obviously, rigorous testing of this hypothesis requires an assessment of the genetic resources present before supplementation begins. **Substock** identification in particular is of critical importance, because the design of the facilities will depend on the number of substocks present. WDF has been very active over the last two years in chinook **substock** identification research in the two subbasins, using the technique of horizontal starch-gel electrophoresis (as described by Aebersold et al. 1987). Approximately 1500 adult chinook, primarily from the Yakima **subbasin** have been collected and analyzed by electrophoresis, following sampling plans developed in 1989 and 1990 (Busack and Phelps 1989, 1990). Approaches vary somewhat between spring and fall chinook in the Yakima, and between Yakima and Klickitat spring chinook, but our intent is to sample all possible substocks. Once putative substocks have been identified, they will be sampled electrophoretically through one complete generation. The information gained through this repeated sampling of year-to-year variability, as well as solidifying our knowledge of **substock** structure, will be invaluable for future genetic monitoring.

WDF's genetic analytical capabilities are being put to other uses peripheral to the **substock** identification task. In 1990 smolts migrating past Prosser Dam were analyzed electrophoretically to verify their identification as spring or fall chinook, and we have done simulations of Yakima spring chinook mixed fisheries to start evaluating our ability to separate the substocks in broodstock collection or in fisheries.

SAMPLING

A total of 24 collections of fish were made for genetic analysis in 1989 and 1990 (Table 1). Sampling for the most part was done as per Young **(1988),** and in all but two cases (Prosser smolts: collections **W90DZ** and **W90DY)** adult fish were sampled. Three collections were made at Klickitat hatchery, but otherwise spawned out adults were sampled on the spawning grounds.

The target sample size was 100. This was met in many cases, but in much of the **Naches** drainage of the Yakima **subbasin** fish numbers have been too low in both years to permit collection of 100. Data on 22 collections will be presented in this document. Two 1989 samples, Yakima springs below Roza **(W89BA)** and Yakima falls at

Benton City (W89CA) are too small for allele frequency data to be used with any confidence.

Table 1. Chinook salmon samples collected for WDF YKFP research, 1989-1990. Unless otherwise specified, fish sampled were adults. Numerals in collection codes denote sampling year.

Run Time/ Area Sampled	Collection Code	Fish Sampled Genetically
Spring Chinook		
American R.	W89AG	80
American R.	W90BA	91
Bumping R.	W89AI	33
Bumping R.	W90BJ	32
Liile Naches R.	W89AV	40
Liile Naches R.	W90BH	21
Naches R.	W89AC	59
Naches R.	W90BI	66
Cle Elum R.	W89AX	100
Yakima R. at Easton	W89AY	100
Yakima R. at Easton	W90BS	50
Yakima R. below Roza	W89BA	14
Yakima R. below Roza	W90BR	111
Carson NFH	W89AR	100
Carson at Klickitat H.	W89AT	100
Klickitat H.	W89AS	100
Klickitat H.	W90BG	100
Klickitat R.	W90BF	35
Fall Chinook		
Yakima R. at Benton City	W89CA	6
Yakima R. at Benton City	W90DF	109
Marion Drain	W89BX	101
Marion Drain	W90DG	52
Smolts		
Prosser smolts ≥90mm FL	W90DY	90
Prosser smolts <90mm FL	W90DZ	90

ELECTROPHORETIC METHODS

The electrophoretic protocols followed and list of alleles recognized are presented in Appendices 1 and 2. Locus and allele nomenclature follow the system of Shaklee et al. (1990). All samples except the Prosser smolt samples were screened at 62 loci. In this document, however, data are presented on only 48 loci, including three isolocus systems (sAAT-1,2*, sMDH-A1,2*, and sMDH-

B1,2*). All loci about which there are any uncertainties in scoring variation have been excluded. In addition, alleles were pooled in three cases where some possibility exists of confusing one allele with the other, and absolute identification would require a large series of sample reruns. The following allele pooling was done (mobilities can be found in Appendix 1): 1) at sAH*, *108 pooled with *112; 2) at sIDHP-1*, *72 pooled with *74; and 3) at TPI-2.2*, *102 pooled with *104. One final scoring convention reflected in the results reported here: at two loci- GPIr* and sMEP-2* - the scoring of heterozygotes is ambiguous. Allele frequencies are based on homozygotes only.

Electrophoretic screening was done differently for the Prosser smolt samples (W90DY and W90DZ). The intent with these samples was to use them only in a mixed-fishery analysis with a baseline consisting of Yakima spring and fall chinook (see Marshall et al. 1991 for details of mixed-fishery analysis). Thus they were evaluated only at 22 loci found to be variable in Yakima chinook.

ANALYSES

Standard genetic analyses were carried out using the BIOSYS-1 program (Swofford and Selander 1981) on 20 chinook collections (24 minus the two small collections and the two Prosser smolt collections). The 20 collections were identically invariant at 13 loci: ADA-2*, mAH-3*, CK-Al*, CK-A2*, GPI-A*, G3PDH-4*, mIDHP-1*, mMEP-1*, PGDH*, PGM-2*, TPI-l*, TPI-2*, and TPI-3*. Allele frequencies for the 35 variable loci are presented in Table 2. Standard measures of genetic variability over 46 loci (inclusion of GPIr* and sMEP-2* was not appropriate due to the homozygotes-only scoring convention) are presented in Table 3. Chi-square tests for conformance to Hardy-Weinberg proportions were conducted for all variable loci in all populations, except for GPIr* and sMEP-2*, which would not be expected to conform to Hardy-Weinberg proportions because of scoring conventions. Of the approximately 400 tests conducted, eleven were significant ($p<0.05$). Since 5% of the tests would be expected to be significant by chance alone, this is an extremely low rate, indicating overall high quality of scoring. One systematic problem was noted, however. Four of the eleven significant tests were at sAAT-4*, and in all four cases heterozygote deficiencies were the cause. Heterozygotes are difficult to distinguish at this locus, so this result is not surprising. The overall variability at this locus was low, so the small amount of misscoring or zero scores that may have occurred will have a negligible impact on allele frequencies.

Table 2. Allele frequencies at 35 variable loci in 20 WDF YXFP chinook collections made in 1989 and 1990.

	COLLECTIONS 1 through 9								
LOCUS	AMER 89	AMER 90	BUM-P 89	BUMP 90	LNACH 89	LNACH 90	NACH 89	NACH 90	CELUM 89
sAAT-1,2*									
(N)	80	91	33	32	40	21	59	66	100
A	1.000	1.000	0.992	1.000	1.000	1.000	1.000	0.996	1.000
B	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.004	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT-3*									
(N)	79	91	33	32	38	21	59	66	99
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT-4*									
(N)	75	77	32	29	36	18	59	46	96
A	1.000	1.000	0.984	0.983	0.958	0.972	0.958	0.989	0.979
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.016	0.017	0.042	0.028	0.042	0.011	0.021
mAAT-1*									
(N)	80	90	33	32	40	21	59	66	100
A	0.987	0.994	0.985	1.000	0.987	0.976	0.983	0.977	0.965
B	0.012	0.006	0.015	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.012	0.024	0.017	0.023	0.035
ADA-l*									
(N)	80	87	32	32	40	21	59	66	100
A	1.000	1.000	0.984	1.000	0.987	0.976	1.000	0.985	0.950
B	0.000	0.000	0.016	0.000	0.012	0.024	0.000	0.015	0.050
sAH*									
(N)	80	90	33	32	39	20	59	59	100
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	0.990
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.010
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mAH-4*									
(N)	80	91	32	32	40	20	59	65	100
A	0.981	0.989	0.969	0.969	0.975	0.950	0.907	0.962	0.835
B	'0.019	0.011	0.031	0.031	0.025	0.050	0.093	0.038	0.165

Table 2. (cont.)

	COLLECTIONS 1 through 9								
LOCUS	AMER 89	AMER 90	BUMP 89	BUMP 90	LNACH 89	LNACH 90	NACH 89	NACH 90	CELUM 89
GPI-B1*									
(N)	80	90	33	32	39	21	59	66	**100**
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.000**
GPI-B2*									
(N)	80	90	33	32	39	21	59	66	100
A	0.900	0.878	0.939	0.906	0.872	0.786	0.831	0.795	0.930
B	0.100	0.122	0.061	0.094	0.128	0.214	0.169	0.205	0.070
GPIr*									
(N)	80	90	33	32	39	21	59	66	100
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GR*									
(N)	80	91	33	32	40	21	59	66	100
A	1.000	1.000	1.000	1.000	1.000	1.000	0.992	0.992	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.008	0.000
HAGH*									
(N)	80	90	33	32	40	21	59	66	100
A	0.662	0.806	0.758	0.828	0.912	0.857	0.881	0.788	0.950
B	0.337	0.194	0.242	0.172	0.087	0.143	0.119	0.212	0.050
mIDHP-2*									
(N)	80	91	33	32	40	21	59	66	100
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sIDHP-1*									
(N)	80	91	33	32	40	21	59	66	100
A	0.862	0.797	0.758	0.875	0.837	0.881	0.856	0.841	0.890
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.137	0.203	0.242	0.125	0.162	0.095	0.136	0.159	0.110
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.024	0.008	0.000	0.000
sIDHP-2*									
(N)	80	91	33	32	40	21	59	66	100
A	0.994	0.995	1.000	0.984	1.000	0.952	0.975	0.985	0.995
B	0.006	0.005	0.000	0.016	0.000	0.048	0.025	0.015	0.005

Table 2. (cont.)

	COLLECTIONS 1 through 9								
LOCUS	AMER 89	AMER 90	BUMP 89	BUMP 90	LNACH 89	LNACH 90	NACH 89	NACH 90	CELUM 89
LDH-B2*									
(N)	80	91	33	32	40	21	59	66	100
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-C*									
(N)	80	91	33	32	40	21	59	66	100
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sMDH-A1,2*									
(N)	80	91	33	32	40	21	59	66	100
A	1.000	1.000	0.992	1.000	0.994	1.000	0.996	0.988	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.008	0.000	0.006	0.000	0.004	0.012	0.000
sMDH-B1,2*									
(N)	80	91	33	32	40	21	59	66	100
A	1.000	1.000	0.976	0.976	0.988	0.988	0.974	0.969	1.000
B	0.000	0.000	0.008	0.016	0.006	0.000	0.009	0.000	0.000
C	0.000	0.000	0.008	0.008	0.006	0.012	0.017	0.031	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.000
mMDH-2*									
(N)	79	90	33	32	40	21	59	64	100
A	0.886	0.900	0.909	0.875	0.912	0.976	0.907	0.914	0.910
B	0.114	0.100	0.091	0.125	0.087	0.024	0.093	0.086	0.090
sMEP-1*									
(N)	80	87	33	31	40	20	58	64	100
A	0.056	0.069	0.045	0.048	0.062	0.050	0.121	0.102	0.160
B	0.944	0.931	0.955	0.952	0.937	0.950	0.879	0.898	0.840
sMEP-2*									
(N)	78	88	33	32	40	21	59	66	100
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

'Table 2. (cont.)

COLLECTIONS 1 through 9

LOCUS	AMER 89	AMER 90	BUMP 89	BUMP 90	LNACH 89	LNACH 90	NACH 89	NACH 90	CELUM 89
MPI*									
(N)	80	90	33	32	40	21	59	66	100
A	0.794	0.678	0.773	0.625	0.650	0.619	0.678	0.697	0.875
B	0.206	0.322	0.227	0.375	0.350	0.381	0.322	0.303	0.125
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1*									
(N)	80	90	32	31	40	21	59	66	100
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGK-2*									
(N)	80	91	31	32	40	21	59	65	100
A	0.475	0.396	0.226	0.312	0.225	0.310	0.220	0.192	0.150
B	0.525	0.604	0.774	0.687	0.775	0.690	0.780	0.808	0.850
PEPA*									
(N)	80	90	31	32	40	19	59	61	100
A	1.000	0.994	1.000	0.984	0.987	1.000	0.992	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.006	0.000	0.016	0.012	0.000	0.008	0.000	0.000
PEPB-1*									
(N)	80	91	33	32	40	21	59	66	100
A	0.987	0.967	0.864	0.844	0.900	0.881	0.915	0.833	0.785
B	0.000	0.016	0.030	0.062	0.037	0.024	0.034	0.068	0.070
C	0.012	0.016	0.106	0.094	0.062	0.095	0.051	0.098	0.145
PEPD-2*									
(N)	80	90	33	32	40	21	59	63	100
A	0.931	0.922	0.955	0.844	0.900	0.929	0.898	0.921	0.915
B	0.069	0.078	0.045	0.156	0.100	0.071	0.102	0.079	0.085
PEP-LT*									
(N)	80	91	33	32	40	21	59	65	100
A	1.000	1.000	0.970	0.969	0.950	0.976	0.992	0.946	0.925
B	0.000	0.000	0.030	0.031	0.050	0.024	0.008	0.054	0.075
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. (cont.)

	COLLECTIONS 1 through 9								
LOCUS	AMER 89	AMER 90	BUMP 89	BUMP 90	LNACH 89	LNACH 90	NACH 89	NACH 90	CELUM 89
sSOD-1*									
(N)	80	91	33	32	40	21	59	64	100
A	0.731	0.769	0.727	0.766	0.687	0.762	0.763	0.781	0.770
B	0.269	0.231	0.273	0.234	0.312	0.238	0.237	0.219	0.230
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mSOD*									
(N)	80	90	33	32	40	2 1	58	66	100
A	3.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
TPI-4*									
(N)	80	91	33	32	40	21	59	66	100
A	0.987	0.989	0.970	0.984	1.000	0.976	0.992	1.000	0.990
B	0.012	0.011	0.030	0.016	0.000	0.024	0.008	0.000	0.010

Table 2.(cont.)

	COLLECTIONS 10 through 18								
LOCUS	Y/EAST 89	Y/EAST 90	YBROZA 90	Y/FALL 90	MARDRN 89	MARDRN 90	CARS 89	CARS/KL 89	KLICKH 89
sAAT-1,2*									
(N)	100	50	111	109	101	52	100	100	100
A	1.000	1.000	1.000	1.000	1.000	1.000	0.992	0.995	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.005	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT-3*									
(N)	99	49	111	109	100	49	98	99	99
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
sAAT-4*									
(N)	95	34	99	105	101	48	88	82	89
A	0.932	0.971	0.929	0.981	0.990	0.990	0.932	0.939	0.972
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.068	0.029	0.071	0.019	0.010	0.010	0.068	0.061	0.028
mAAT-1*									
(N)	100	50	111	108	101	52	100	100	100
A	0.945	0.930	0.910	0.991	0.985	0.962	0.985	0.975	0.995
B	0.000	0.000	0.000	0.005	0.010	0.038	0.000	0.000	0.000
C	0.055	0.070	0.090	0.005	0.005	0.000	0.015	0.025	0.005
ADA-l*									
(N)	100	50	111	109	101	52	100	100	100
A	0.935	0.940	0.968	0.991	0.990	0.971	0.980	0.965	0.960
B	0.065	0.060	0.032	0.009	0.010	0.029	0.020	0.035	0.040
sAH*									
(N)	100	48	111	109	101	52	100	100	100
A	0.980	0.969	0.995	0.784	0.901	0.942	1.000	1.000	0.985
B	0.020	0.031	0.005	0.206	0.084	0.038	0.000	0.000	0.015
C	0.000	0.000	0.000	0.009	0.015	0.019	0.000	0.000	0.000
mAH-4*									
(N)	100	50	111	109	100	52	100	100	100
A	0.885	0.870	0.878	0.904	0.835	0.779	0.975	0.980	0.980
B	0.115	0.130	0.122	0.096	0.165	0.221	0.025	0.020	0.020
GPI-B1*									
(N)	100	50	111	109	101	52	100	99	100
A	1.000	1.000	1.000	0.995	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000

Table 2.(cont.)

	COLLECTIONS 10 through 18								
LOCUS	Y/EAST 89	Y/EAST 90	YBROZA 90	Y/FALL 90	MARDRN 89	MARDRN 90	CARS 89	CARS/KL 89	XLICKH 89
GPI-B2*									
(N)	100	50	111	109	101	52	100	99	100
A	0.975	0.970	0.973	0.959	0.950	0.962	0.980	0.995	0.995
B	0.025	0.030	0.027	0.041	0.050	0.038	0.020	0.005	0.005
GPIr*									
(N)	100	50	111	109	101	52	100	100	100
A	1.000	1.000	1.000	0.972	1.000	0.962	1.000	1.000	1.000
B	0.000	0.000	0.000	0.028	0.000	0.038	0.000	0.000	0.000
GR*									
(N)	100	50	111	109	101	52	100	100	100
A	1.000	1.000	0.995	0.986	0.995	1.000	0.990	1.000	0.890
B	0.000	0.000	0.005	0.014	0.005	0.000	0.010	0.000	0.110
HAGH*									
(N)	100	50	111	109	101	52	100	100	100
A	0.940	0.980	0.964	0.991	1.000	1.000	0.915	0.850	0.915
B	0.060	0.020	0.036	0.009	0.000	0.000	0.085	0.150	0.085
mIDHP-2*									
(N)	100	50	111	109	101	52	99	100	100
A	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
sIDHP-1*									
(N)	100	50	111	109	101	52	100	100	100
A	0.875	0.950	0.905	1.000	0.995	1.000	0.760	0.830	0.935
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.125	0.050	0.095	0.000	0.005	0.000	0.190	0.165	0.065
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.005	0.000
sIDHP-2*									
(N)	100	50	111	109	101	52	100	100	100
A	0.975	1.000	0.995	0.867	0.891	0.798	0.995	1.000	0.965
B	0.025	0.000	0.005	0.133	0.109	0.202	0.005	0.000	0.035
LDH-B2*									
(N)	100	50	111	109	101	52	100	100	100
A	0.985	1.000	1.000	0.991	1.000	1.000	0.980	0.990	1.000
B	0.015	0.000	0.000	0.005	0.000	0.000	0.020	0.010	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000

Table 2.(cont.)

	COLLECTIONS 10 through 18								
LOCUS	Y/EAST 89	Y/EAST 90	YBROZA 90	Y/FALL 90	MARDRN 89	MARDRN 90	CARS 89	CARS/KL 89	KLICKH 89
LDH-C*									
(N)	100	49	**111**	**109**	99	**51**	**100**	99	100
A	1.000	1.000	**1.000**	**0.991**	**1.000**	**1.000**	**1.000**	**1.000**	0.945
B	0.000	0.000	**0.000**	**0.009**	**0.000**	**0.000**	**0.000**	**0.000**	0.000
C	0.000	0.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.055**
sMDH-A1,2*									
(N)	**100**	**50**	**111**	**109**	**101**	**52**	100	100	**100**
A	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	1.000	1.000	**1.000**
B	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.000	0.000	**0.000**
C	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.000	0.000	**0.000**
sMDH-B1,2*									
(N)	**100**	**50**	**111**	109	**101**	**52**	100	100	**100**
A	**0.995**	**0.995**	**0.997**	0.974	**0.955**	**0.976**	0.967	0.977	**0.990**
B	**0.000**	**0.000**	**0.000**	**0.016**	**0.005**	**0.005**	0.000	0.000	**0.010**
C	**0.005**	**0.000**	**0.003**	**0.007**	**0.040**	**0.019**	0.000	0.000	**0.000**
D	0.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.000	0.000	**0.000**
E	0.000	**0.005**	**0.000**	**0.003**	**0.000**	**0.000**	**0.033**	0.013	**0.000**
mMDH-2*									
(N)	100	**50**	**111**	109	**101**	**52**	**99**	100	**100**
A	0.915	**0.870**	**0.919**	0.982	**0.990**	**0.990**	**0.793**	**0.745**	**0.760**
B	**0.085**	**0.130**	**0.081**	0.018	**0.010**	**0.010**	**0.207**	**0.255**	**0.240**
sMEP-1*									
(N)	**100**	**48**	**111**	109	**100**	**52**	**100**	**100**	**100**
A	**0.245**	**0.229**	**0.144**	**0.784**	**0.810**	**0.750**	**0.075**	**0.080**	**0.225**
B	**0.755**	**0.771**	**0,856**	**0.216**	**0.190**	**0.250**	**0.925**	**0.920**	**0.775**
sMEP-2*									
(N)	**100**	**50**	**109**	**106**	**100**	**51**	**97**	**91**	**92**
A	1.000	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.989**	**1.000**
B	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.011**	**0.000**
MPI*									
(N)	**100**	**50**	**111**	**109**	**101**	**52**	**100**	**100**	**100**
A	**0.825**	**0.890**	**0.892**	**0.670**	**0.856**	**0.904**	0.910	**0.895**	**0.850**
B	**0.175**	0.110	**0.108**	**0.330**	**0.139**	**0.096**	0.090	0.105	**0.120**
C	**0.000**	0.000	**0.000**	**0.000**	**0.005**	**0.000**	0.000	0.000	**0.030**
PGM-1*									
(N)	**100**	**50**	**111**	**109**	**101**	**52**	100	100	**100**
A	**0.985**	**1.000**	**0.995**	**1.000**	**1.000**	**1.000**	1.000	1.000	**1.000**
B	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.000	0.000	**0.000**
C	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.000	0.000	**0.000**
D	**0.015**	**0.000**	**0.005**	**0.000**	**0.000**	**0.000**	0.000	0.000	**0.000**

Table 2.(cont.)

	COLLECTIONS 10 through 18								
LOCUS	y/EAST 89	Y/EAST 90	YBROZA 90	Y/FALL 90	MARDRN 89	MARDRN 90	CARS 89	CARS/KL 89	KLICKH 89
PGK-2*									
(N)	100	50	111	109	101	52	100	100	100
A	0.185	0.260	0.144	0.528	0.579	0.490	0.130	0.150	0.210
B	0.815	0.740	0.856	0.472	0.421	0.510	0.870	0.850	0.790
PEPA*									
(N)	98	50	111	109	101	51	100	100	100
A	0.995	1.000	0.986	0.963	0.936	0.951	1.000	0.990	1.000
B	0.000	0.000	0.000	0.037	0.064	0.049	0.000	0.000	0.000
C	0.005	0.000	0.014	0.000	0.000	0.000	0.000	0.010	0.000
PEPB-1*									
(N)	100	50	111	109	100	52	100	100	100
A	0.745	0.810	0.829	0.784	0.950	0.913	0.810	0.780	0.920
B	0.125	0.080	0.054	0.206	0.040	0.067	0.115	0.070	0.030
C	0.130	0.110	0.117	0.009	0.010	0.019	0.075	0.150	0.050
PEPD-2*									
(N)	100	50	111	109	101	52	100	100	100
A	0.940	0.890	0.914	0.982	1.000	1.000	0.995	1.000	0.990
B	0.060	0.110	0.086	0.018	0.000	0.000	0.005	0.000	0.010
PEP-LT*									
(N)	100	50	111	109	101	52	100	100	100
A	0.965	0.900	0.878	0.752	0.891	0.952	0.955	0.965	0.990
B	0.035	0.100	0.122	0.248	0.104	0.048	0.045	0.035	0.010
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
sSOD-1*									
(N)	100	50	111	109	101	52	100	100	100
A	0.805	0.760	0.847	0.560	0.634	0.721	0.845	0.845	0.685
B	0.195	0.240	0.153	0.436	0.361	0.279	0.155	0.155	0.315
C	0.000	0.000	0.000	0.005	0.005	0.000	0.000	0.000	0.000
mSOD*									
(N)	100	50	111	109	101	52	100	100	100
A	0.995	1.000	0.986	1.000	1.000	1.000	0.995	1.000	1.000
B	0.005	0.000	0.014	0.000	0.000	0.000	0.005	0.000	0.000
TPI-4*									
(N)	100	50	111	109	100	52	100	100	100
A	0.990	0.970	0.950	0.982	0.990	1.000	0.955	0.875	0.990
B	0.010	0.030	0.050	0.018	0.010	0.000	0.045	0.125	0.010

Table 2.(cont.)

	COLLECTIONS 19 & 20	
LOCUS	KLICKH 90	KLICKR 90
sAAT-1,2*		
(N)	100	35
A	0.992	0.978
B	0.008	0.007
C	0.000	0.014
sAAT-3*		
(N)	100	34
A	1.000	1.000
B	0.000	0.000
C	0.000	0.000
sAAT-4*		
(N)	91	29
A	1.000	1.000
B	0.000	0.000
C	0.000	0.000
mAAT-1*		
(N)	100	35
A	0.985	0.971
B	0.000	0.000
C	0.015	0.029
ADA-l*		
(N)	100	35
A	0.975	0.986
B	0.025	0.014
sAH*		
(N)	97	34
A	0.985	0.956
B	0.015	0.044
C	0.000	0.000
mAH-4*		
(N)	100	35
A	0.950	0.886
B	0.050	0.114
GPI-B1*		
(N)	100	35
A	1.000	1.000
B	0.000	0.000

Table 2.(cont.)

	COLLECTIONS 19 & 20	
LOCUS	KLICKH 89	KLICKR 90
GPI-B2*		
(N)	99	34
A	0.995	0.985
B	0.005	0.015
GPIr*		
(N)	100	35
A	1.000	1.000
B	0.000	0.000
GR*		
(N)	100	35
A	0.890	0.900
B	0.110	0.100
HAGH*		
(N)	99	34
A	0.874	0.897
B	0.126	0.103
mIDHP-2*		
(N)	100	35
A	1.000	1.000
B	0.000	0.000
sIDHP-1*		
(N)	99	35
A	0.843	0.914
B	0.000	0.000
C	0.152	0.071
D	0.000	0.000
E	0.000	0.000
F	0.005	0.014
sIDHP-2*		
(N)	99	35
A	0.944	0.957
B	0.056	0.043
LDH-B2*		
(N)	100	35
A	1.000	1.000
B	0.000	0.000
C	0.000	0.000
D	0.000	0.000

Table 2.(cont.)

LOCUS	COLLECTIONS 19 & 20 KLICKH 90	KLICKR 90
LDH-C*		
(N)	100	34
A	0.990	1.000
B	0.000	0.000
C	0.010	0.000
sMDH-A1,2*		
(N)	100	35
A	1.000	**1.000**
B	0.000	**0.000**
C	0.000	**0.000**
sMDH-B1,2*		
(N)	100	35
A	0.990	0.964
B	0.010	0.029
C	0.000	0.007
D	0.000	0.000
E	0.000	0.000
mMDH-2*		
(N)	100	35
A	0.795	0.729
B	0.205	0.271
sMEP-1*		
(N)	100	35
A	0.255	0.429
B	0.745	0.571
sMEP-2*		
(N)	99	35
A	1.000	0.971
B	0.000	0.029
MPI*		
(N)	100	34
A	0.825	0.750
B	0.165	0.221
C	0.010	0.029
PGM-1*		
(N)	100	33
A	1.000	1.000
B	0.000	0.000
C	0.000	0.000
D	0.000	0.000

Table 2.(cont.)

LOCUS	COLLECTIONS 19 & 20 KLICKH 89	KLICKR 90
PGK-2*		
(N)	100	35
A	0.265	0.243
B	0.735	0.757
PEPA*		
(N)	100	35
A	0.990	0.957
B	0.010	0.043
C	0.000	0.000
PEPB-1*		
(N)	100	35
A	0.915	0.900
B	0.045	0.057
C	0.040	0.043
PEPD-2*		
(N)	100	35
A	0.995	0.986
B	0.005	0.014
PEP-LT*		
(N)	100	35
A	0.985	0.986
B	0.015	0.014
C	0.000	0.000
D	0.000	0.000
sSOD-1*		
(N)	100	34
A	0.660	0.706
B	0.340	0.294
C	~0.000	0.000
mSOD*		
(N)	100	34
A	1.000	0.985
B	0.000	0.015
TPI-4*		
(N)	100	35
A	0.975	0.957
B	0.025	0.043

Table 3. Measures of genetic variability in YKFP spring and fall chinook stocks, evaluated over 46 loci in 20 collections made in 1989 and 1990. Standard errors are in parentheses.

Collection	Mean Sample Size per Locus	Mean Alleles per Locus	Percentage of Loci Polymorphic *	Mean Heterozygosity Direct-Count	Hdywbg Exp. **
SPRING CHINOOK					
1. AMERICAN R.89	79.8 (0.1)	1.3 (0.1)	19.6	0.062 (0.021)	0.058 (0.019)
2. AMERICAN R. 90	89.8 (0.4)	1.3 (0.1)	19.6	0.057 (0.018)	0.059 (0.019)
3. BUMPING R. 89	32.7 (0.1)	1.5 (0.1)	17.4	0.067 (0.018)	0.065 (0.018)
4. BUMPING R. 90	31.8 (0.1)	1.4 (0.1)	19.6	0.069 (0.019)	0.069 (0.019)
5. L NACHES 89	39.7 (0.1)	1.4 (0.1)	23.9	0.060 (0.016)	0.064 (0.017)
6.L. NACHES 90	20.8 (0.1)	1.4 (0.1)	21.7	0.068 (0.018)	0.067 (0.018)
7. NACHES R.89	58.9 (0.0)	1.5 (0.1)	26.1	0.071 (0.018)	0.068 (0.017)
8. NACHES R. 90	64.8 (0.5)	1.5 (0.1)	26.1	0.072 (0.017)	0.073 (0.018)
9. CLE ELUM 89	99.9 (0.1)	1.5 (0.1)	28.3	0.063 (0.016)	0.083 (0.015)
10. YAKIMA/EASTON 89	99.8 (0.1)	1.5 (0.1)	28.3	0.068 (0.017)	0.069 (0.016)
11. YAKIMA/EASTON 90	49.2 (0.5)	1.4 (0.1)	26.1	0.069 (0.017)	0.068 (0.017)
12. YAKIMA RIVER BELOWROZA90	110.7 (0.3)	1.5 (0.1)	26.1	0.060 (0.013)	0.062 (0.014)
13. CARSON NFH 89	99.7 (0.3)	1.5 (0.1)	21.7	0.058 (0.015)	0.059 (0.015)
14. CARSON AT KLICKITAT H.	99.5 (0.4)	1.5 (0.1)	21.7	0.062 (0.016)	0.062 (0.016)

Table 3. (cont.1

Collection	Mean Sample Size per Locus	Mean Alleles per Locus	Percentage of Loci Polymorphic *	Mean Heterozygosity Direct-Count	Hdywbg Exp. • *
15. KLICKITAT H 89	99.7 (0.2)	1.5 (0.1)	21.7	0.061 (0.016)	0.062 (0.017)
16. KLICKITAT H 90	99.6 (0.2)	1.5 (0.1)	23.9	0.065 (0.016)	0.070 (0.018)
17. KLICKITAT R. 90	34.6 (0.1)	1.6 (0.1)	23.9	0.080 (0.019)	0.082 (0.020)
FALL CHINOOK					
18. YAKIMA RIVER 90	107.9 (1.0)	1.7 (0.1)	21.7	0.082 (0.022)	0.082 (0.022)
19. MARION DRAIN 89	99.9 (1.0)	1.6 (0.1)	23.9	0.066 (0.018)	0.065 (0.018)
20. MARION DRAIN 90	51.5 (0.3)	1.4 (0.1)	17.4	0.084 (0.019)	0.084 (0.019)

* A LOCUS IS CONSIDERED POLYMORPHIC IF THE FREQUENCY OF THE MOST COMMON ALLELE DOES NOT EXCEED 0.95

• * UNBIASED ESTIMATE (SEE NEI, 1978)

Genetic distance statistics were calculated among all pairwise combinations of the 20 collections to gain insight into the genetic relationships among the stocks. Two statistics were used, the unbiased genetic distance of Nei (1978) (Table 4) and the chord distance of Cavalli-Sforza and Edwards (1967) (Table 5). The Cavalli-Sforza and Edwards distances will hereafter be referred to as CSE distances. The genetic distances are summarized graphically in dendrograms generated by the unweighted pair-group method (Sneath and Sokal 1973) (Figs. 1 and 2). The two methods are included to provide perspective on the use of genetic distance statistics. There are many such statistics. Although Nei's statistics are the most used, they have been criticized for dependency on polymorphism (Hillis 1984). The CSE statistic offers an alternative which relies on a more geometric, but possibly less genetic (Weir 1990) approach. Similarly, there are many clustering algorithms for generating dendrograms available. The unweighted pair-group method we have used here is the most common, but not necessarily the "best".

Table 4. Nei's unbiased genetic distances, evaluated over 48 loci, among 20 WDF YKFP chinook salmon collections made in 1989-1990.

	Collection	1	2	3	4	5	6	7	8
1	AMERICAN R. 89	*****	0.001	0.002	0.002	0.003	0.002	0.003	0.003
2	AMERICAN R. 90	0.001	*****	0.001	0.000	0.001	0.000	0.001	0.001
3	BUMPING R. 89	0.002	0.001	*****	0.000	0.000	0.001	0.001	0.000
4	BUMPING R. 90	0.002	0.000	0.000	*****	0.000	0.000	0.000	0.000
5	L. NACHES 89	0.003	0.001	0.000	0.000	*****	0.000	0.000	0.000
6	L. NACHES 90	0.002	0.000	0.001	0.000	0.000	*****	0.000	0.000
7	NACHES R. 89	0.003	0.001	0.001	0.000	0.000	0.000	*****	0.000
8	NACHES R. 90	0.003	0.001	0.000	0.000	0.000	0.000	0.000	*****
9	CLE ELTJM 89	0.006	0.004	0.002	0.003	0.002	0.003	0.002	0.002
10	YAK/EASTON 89	0.006	0.004	0.002	0.003	0.002	0.003	0.002	0.002
11	YAK/EASTON 90	0.006	0.004	0.003	0.003	0.003	0.003	0.002	0.003
12	YAK BELOW ROZA 90	0.007	0.005	0.003	0.003	0.003	0.004	0.002	0.003
13	YAKIMA FALL 90	0.021	0.020	0.022	0.019	0.019	0.019	0.018	0.020
14	MARION DRAIN 89	0.019	0.019	0.021	0.020	0.019	0.019	0.018	0.020
15	MARION DRAIN 90	0.017	0.017	0.018	0.018	0.017	0.017	0.015	0.017
16	CARSON NFH 89	0.006	0.004	0.002	0.004	0.003	0.004	0.003	0.003
17	CARSON/KLICK 89	0.006	0.005	0.002	0.003	0.004	0.005	0.003	0.003
18	KLICRITAT H 89	0.005	0.004	0.003	0.004	0.003	0.004	0.003	0.004
19	KLICKITAT H 90	0.004	0.003	0.002	0.003	0.003	0.004	0.002	0.003
20	KLICKITAT R. 90	0.007	0.006	0.005	0.005	0.005	0.006	0.004	0.005

Table 4.(cont.)

	Collection	9	10	11	12	13	14	15	16
1	AMERICAN R. 89	0.006	0.006	0.006	0.007	0.021	0.019	0.017	0.006
2	AMERICAN R. 90	0.004	0.004	0.004	0.005	0.020	0.019	0.017	0.004
3	BUMPING R. 89	0.002	0.002	0.003	0.003	0.022	0.021	0.018	0.002
4	BUMPING R. 90	0.003	0.003	0.003	0.003	0.019	0.020	0.018	0.004
5	L. NACHES 89	0.002	0.002	0.003	0.003	0.019	0.019	0.017	0.003
6	L. NACHES 90	0.003	0.003	0.003	0.004	0.019	0.019	0.017	0.004
7	NACHES R. 89	0.002	0.002	0.002	0.002	0.018	0.018	0.015	0.003
8	NACHES R. 90	0.002	0.002	0.003	0.003	0.020	0.020	0.017	0.003
9	CLE ELUM 89	*****	0.000	0.000	0.000	0.018	0.016	0.013	0.002
10	YAK/EASTON 89	0.000	*****	0.000	0.001	0.015	0.014	0.011	0.002
11	YAK/EASTON 90	0.000	0.000	*****	0.000	0.014	0.012	0.010	0.002
12	YAK BELOW ROZA 90	0.000	0.001	0.000	*****	0.019	0.018	0.014	0.001
13	YAKIMA FALL 90	0.018	0.015	0.014	0.019	*****	0.002	0.004	0.024
14	MARION DRAIN 89	0.016	0.014	0.012	0.018	0.002	*****	0.001	0.022
15	MARION DRAIN 90	0.013	0.011	0.010	0.014	0.004	0.001	*****	0.018
16	CARSON NFH 89	0.002	0.002	0.002	0.001	0.024	0.022	0.018	*****
17	CARSON/KLICK 89	0.002	0.002	0.003	0.002	0.024	0.023	0.019	0.000
18	KLICKITAT H 89	0.002	0.002	0.002	0.003	0.016	0.014	0.012	0.002
19	KLICKITAT H 90	0.002	0.002	0.002	0.003	0.014	0.012	0.010	0.003
20	KLICKITAT R. 90	0.003	0.002	0.002	0.004	0.010	0.009	0.007	0.005

Table 4. (cont.)

	Collection	17	18	19	20
1	AMERICAN R. 89	0.006	0.005	0.004	0.007
2	AMERICAN R. 90	0.005	0.004	0.003	0.006
3	BUMPING R. 89	0.002	0.003	0.002	0.005
4	BUMPING R. 90	0.003	0.004	0.003	0.005
5	L. NACHES 89	0.004	0.003	0.003	0.005
6	L. NACHES 90	0.005	0.004	0.004	0.006
7	NACHES R. 89	0.003	0.003	0.002	0.004
8	NACHES R. 90	0.003	0.004	0.003	0.005
9	CLE ELUM 89	0.002	0.002	0.002	0.003
10	YAK/EASTON 89	0.002	0.002	0.002	0.002
11	YAK/EASTON 90	0.003	0.002	0.002	0.002
12	YAK BELOW ROZA 90	0.002	0.003	0.003	0.004
13	YAKIMA FALL 90	0.024	0.016	0.014	0.010
14	MARION DRAIN 89	0.023	0.014	0.012	0.009
15	MARION DRAIN 90	0.019	0.012	0.010	0.007
16	CARSON NFH 89	0.000	0.002	0.003	0.005
17	CARSON/KLICK 89	*****	0.002	0.003	0.005
18	KLICKITAT H 89	0.002	*****	0.000	0.001
19	KLICKITAT H 90	0.003	0.000	*****	0.001
20	KLICKITAT R. 90	0.005	0.001	0.001	*****

Table 5. Cavalli-Sforza and Edwards chord distances, evaluated over 48 loci, among 20 WDF YKFP chinook collections made in 1989-1990.

Collection	1	2	3	4	5	6	7	8
1 AMERICAN R. 89	*****	0.028	0.052	0.055	0.064	0.062	0.061	0.066
2 AMERICAN R. 90	0.028	*****	0.047	0.041	0.048	0.052	0.048	0.056
3 BUMPING R. 89	0.052	0.047	*****	0.043	0.043	0.054	0.051	0.047
4 BUMPING R. 90	0.055	0.041	0.043	*****	0.035	0.045	0.036	0.046
5 L. NACHES 89	0.064	0.048	0.043	0.035	*****	0.044	0.035	0.039
6 L. NACHES 90	0.062	0.052	0.054	0.045	0.044	*****	0.040	0.043
7 NACHES R. 89	0.061	0.048	0.051	0.036	0.035	0.040	*****	0.039
8 NACHES R. 90	0.066	0.056	0.047	0.046	0.039	0.043	0.039	*****
9 CLEELUM89	0.084	0.076	0.060	0.063	0.054	0.061	0.055	0.054
10 YAKIMA/EASTON 89	0.091	0.081	0.067	0.068	0.061	0.066	0.059	0.059
11 YAKIMA/EASTON 90	0.091	0.085	0.073	0.073	0.065	0.074	0.068	0.068
12 YAK BELOW ROZA 90	0.093	0.085	0.069	0.070	0.062	0.070	0.062	0.066
13 YAKIMA FALL 90	0.141	0.136	0.138	0.128	0.128	0.126	0.123	0.123
14 MARION DRAIN 89	0.131	0.129	0.133	0.129	0.125	0.122	0.117	0.123
15 MARION DRAIN 90	0.132	0.129	0.132	0.129	0.127	0.122	0.117	0.124
16 CARSON NFH 89	0.087	0.080	0.060	0.074	0.070	0.080	0.072	0.072
17 CARSON/KLICK 89	0.087	0.082	0.059	0.076	0.075	0.083	0.078	0.078
18 KLICKITAT H 89	0.083	0.081	0.075	0.079	0.077	0.085	0.073	0.077
19 KLICKITAT H 90	0.077	0.074	0.070	0.076	0.076	0.082	0.070	0.072
20 KLICKITAT R. 90	0.096	0.092	0.089	0.089	0.091	0.095	0.080	0.084

Table 5. (cont.)

	Collection	9	10	11	12	13	14	15	16
1	AMERICAN R. 89	0.084	0.091	0.091	0.093	0.141	0.131	0.132	0.087
2	AMERICAN R. 90	0.076	0.081	0.085	0.085	0.136	0.129	0.129	0.080
3	BUMPING R. 89	0.060	0.067	0.073	0.069	0.138	0.133	0.132	0.060
4	BUMPING R. 90	0.063	0.068	0.073	0.070	0.128	0.129	0.129	0.074
5	L. NACHES 89	0.054	0.061	0.065	0.062	0.128	0.125	0.127	0.070
6	L. NACHES 90	0.061	0.066	0.074	0.070	0.126	0.122	0.122	0.080
7	NACHES R. 89	0.055	0.059	0.068	0.062	0.123	0.117	0.117	0.072
8	NACHES R. 90	0.054	0.059	0.068	0.066	0.123	0.123	0.124	0.072
9	CLE ELUM 89	*****	0.032	0.031	0.032	0.116	0.111	0.108	0.059
10	YAKIMA/EASTON 89	0.032	*****	0.040	0.037	0.109	0.106	0.103	0.060
11	YAKIMA/EASTON 90	0.031	0.040	*****	0.036	0.106	0.102	0.103	0.067
12	YAK BELOW ROZA 90	0.032	0.037	0.036	*****	0.121	0.116	0.115	0.059
13	YAKIMA FALL 90	0.116	0.109	0.106	0.121	*****	0.056	0.067	0.137
14	MARION DRAIN 89	0.111	0.106	0.102	0.116	0.056	*****	0.040	0.133
15	MARION DRAIN 90	0.108	0.103	0.103	0.115	0.067	0.040	*****	0.131
16	CARSON NFH 89	0.059	0.060	0.067	0.059	0.137	0.133	0.131	*****
17	CARSON/KLICK 89	0.065	0.064	0.069	0.061	0.144	0.138	0.137	0.040
18	KLICKITAT H 89	0.070	0.068	0.070	0.073	0.117	0.110	0.111	0.068
19	KLICKITAT H 90	0.069	0.068	0.071	0.075	0.111	0.104	0.106	0.069
20	KLICKITAT R. 90	0.078	0.075	0.077	0.082	0.101	0.095	0.100	0.087

Table 5. (cont.)

	Collection	17	18	19	20
1	AMERICAN R. 89	0.087	0.083	0.077	0.096
2	AMERICAN R. 90	0.082	0.081	0.074	0.092
3	BUMPING R. 89	0.059	0.075	0.070	0.089
4	BUMPING R. 90	0.076	0.079	0.076	0.089
5	L. NACHES 89	0.075	0.077	0.076	0.091
6	L. NACHES 90	0.083	0.085	0.082	0.095
7	NACHES R. 89	0.078	0.073	0.070	0.080
8	NACHES R. 90	0.078	0.077	0.072	0.084
9	CLE ELUM 89	0.065	0.070	0.069	0.078
10	YAKIMA/EASTON 89	0.064	0.068	0.068	0.075
11	YAKIMA/EASTON 90	0.069	0.070	0.071	0.077
12	YAK BELOW ROZA 90	0.061	0.073	0.075	0.082
13	YAKIMA FALL 90	0.144	0.117	0.111	0.101
14	MARION DRAIN 89	0.138	0.110	0.104	0.095
15	MARION DRAIN 90	0.137	0.111	0.106	0.100
16	CARSON NFH 89	0.040	0.068	0.069	0.087
17	CARSON/KLICK 89	*****	0.072	0.073	0.088
18	KLICKITAT H 89	0.072	*****	0.035	0.058
19	KLICKITAT H 90	0.073	0.035	*****	0.044
20	KLICKITAT R. 90	0.088	0.058	0.044	*****

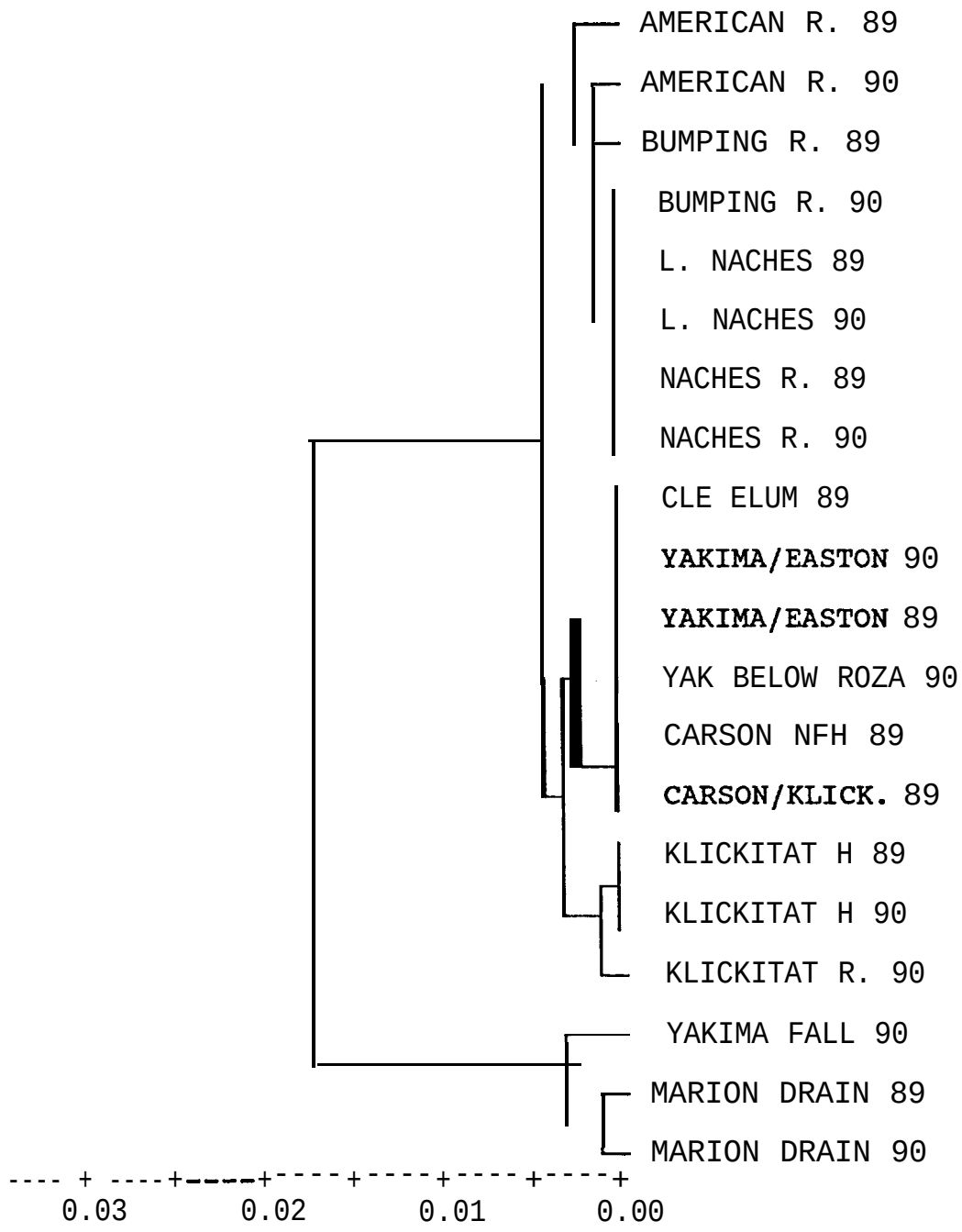

Fig. 1. UPGMA dendrogram of Nei's unbiased genetic distances calculated over 48 loci in 20 YKFP collections of chinook salmon.

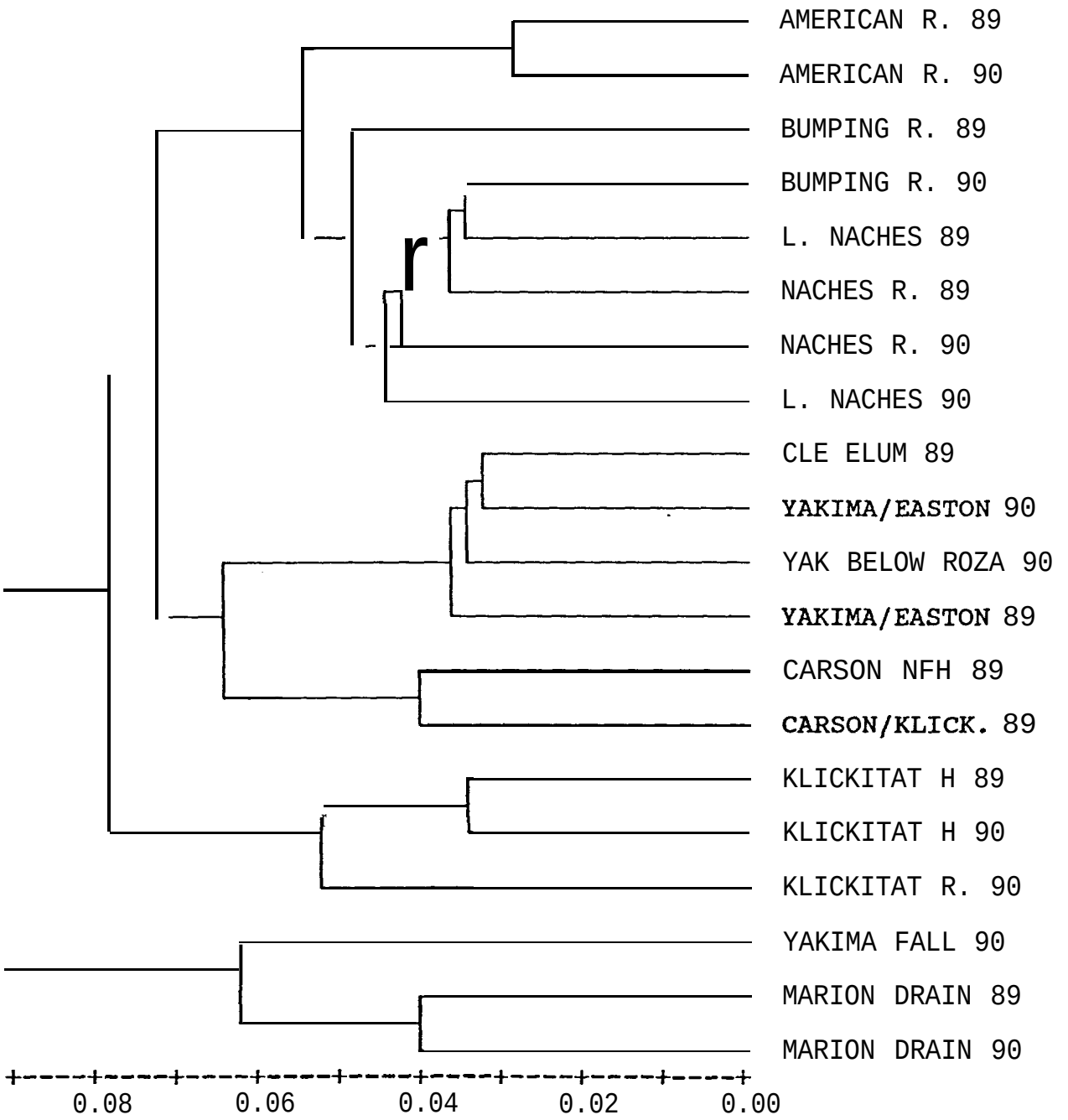

Fig. 2. UPGMA dendrogram of Cavalli-Sforza and Edwards' (1967) chord distances, calculated over 48 loci in 20 collections of chinook salmon. The two unconnected clusters join at a distance of 0.12.

The method of principal coordinates (Gower 1966) was used as an alternative graphical approach to representing the genetic distances among the Yakima spring chinook collections (Figs. 3 and 4). Principal coordinates were derived using NTSYS, version 1.4 (Rohlf 1988), and plotted with Statgraphics (STSC, Inc. 1986).

G-tests (Sokal and Rohlf 1981) of heterogeneity of allele frequencies were done for every pairwise comparison of collections as another means of evaluating genetic differences between collections. The G-test program used was written by R. Waples of the National Marine Fisheries Service, Seattle, and modified for WDF use by C. Busack. Results are presented in Table 6.

Two types of mixed-stock fishery analyses (e.g., Marshall et al. 1991) were carried out on the chinook samples. The Prosser smolt collections were analyzed as fishery samples with an all-Yakima baseline to determine Spring-fall composition, using WDF's MLE (maximum likelihood estimation) program. The program was written by R. Millar. Yakima spring chinook mixed-stock fisheries simulations to evaluate the potential for discriminating between stocks in a mixed group were done using Millar's SIMLE program, also written for WDF. Simulation results are presented in Table 7.

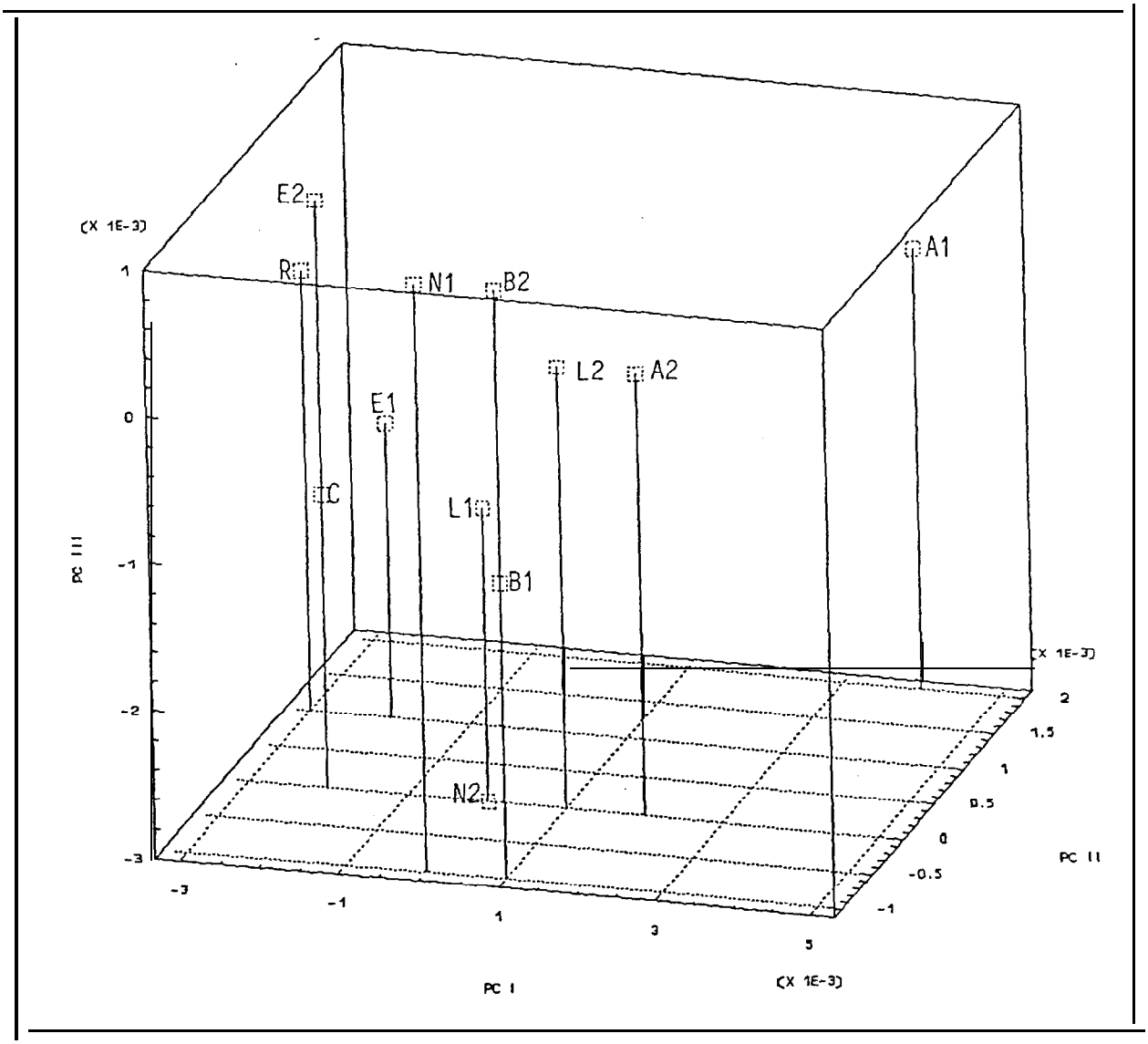

Figure 3. Plot of first three principal coordinates calculated from Nei's unbiased genetic distances over 48 loci among 12 collections of Yakima subbasin spring chinook. Collection label codes are as follows: A, American R.; B, Bumping R.; L, Little Naches R.; N, Naches R.; E, Yakima R. at Easton; C, Cle Elum R. In cases where a site was sampled both in 1989 and 1990, the label "1" denotes the 1989 collection and "2" the 1990 collection.

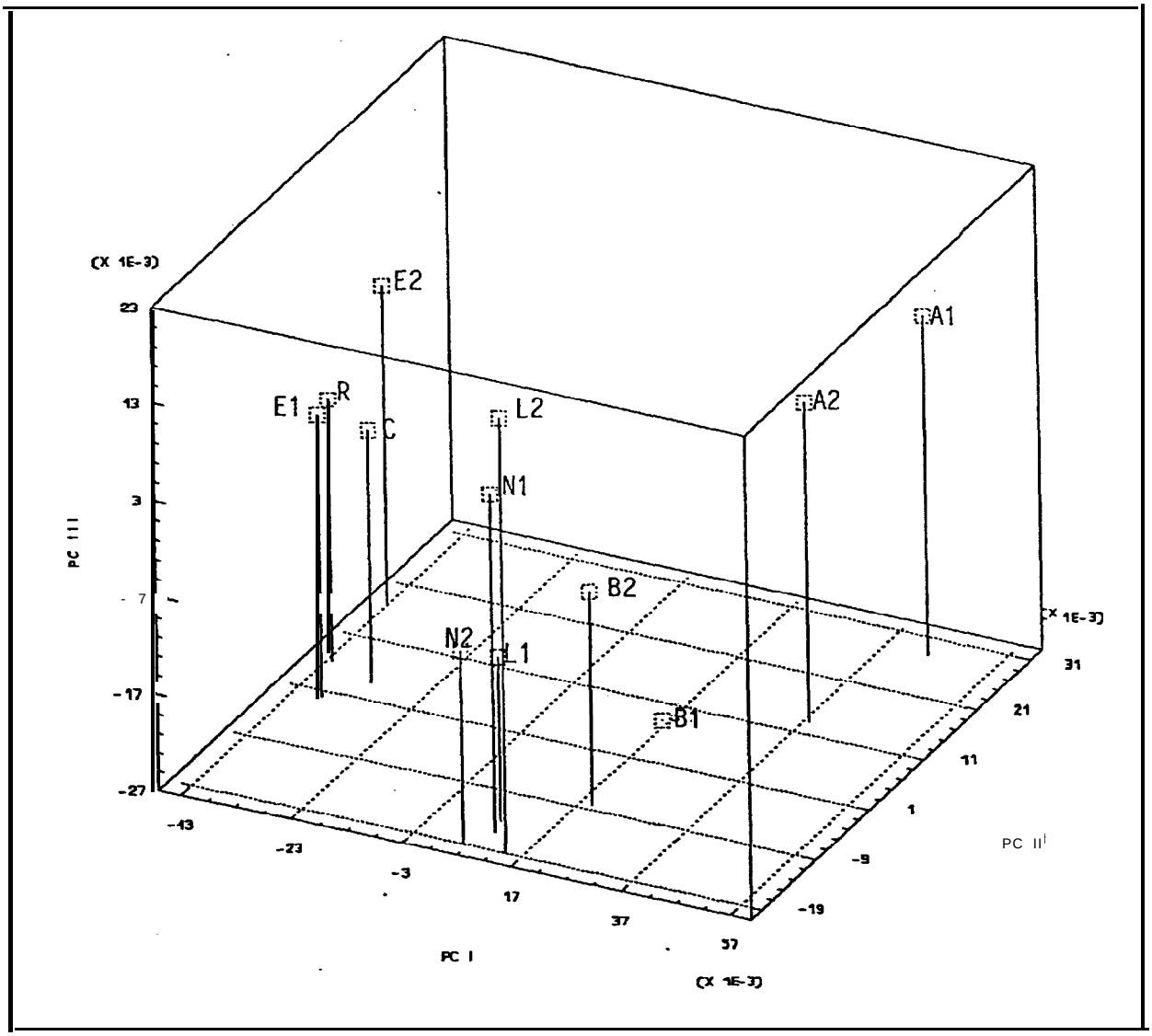

Figure 4. Plot of first three principal coordinates calculated from Cavalli-Sforza and Edwards chord distances over 48 loci among 12 collections of Yakima subbasin spring chinook. Collection label codes are as follows: A, American R.; B, Bumping R.; L, Little Naches R.; N, Naches R.; E, Yakima R. at Easton; C, Cle Elum R. In cases where a site was sampled both in 1989 and 1990, the label "1" denotes the 1989 collection and "2" the 1990 collection.

Table 6. Non-significant G-test results for pairwise comparisons of 20 YKPP chinook collections. All comparisons not shown were significant at the $p \leq 0.01$ level overall.

Comparison	Number of significant ($p \leq 0.05$) single-locus comparisons
A Comparisons not significant overall at $p \leq 0.05$	
BUMPING R. 89 VS BUMPING R. 90	1
BUMPING R. 89 VS L NACHES 89	1
BUMPING R. 89 VS L. NACHES 90	1
BUMPING R. 89 VS NACHES R. 90	1
BUMPING R. 90 VS L NACHES 89	0
BUMPING R. 90 VS L NACHES 90	1
BUMPING R. 90 VS NACHES R. 89	0
BUMPING R. 90 VS NACHES R. 90	2
L NACHES 89 VS L NACHES 90	1
L NACHES 89 VS NACHES R. 89	1
L NACHES 89 VS NACHES R. 90	1
L NACHES 90 VS NACHES R. 89	0
L NACHES 90 VS NACHES R. 90	0
NACHES R. 89 VS NACHES R. 90	2
CLE ELUM 89 VS YAKIMA/EASTON 90	1
YAKIMA/EASTON 90 VS YAK BELOW ROZA 90	1
B. Comparisons significant overall at at $p \leq 0.05$, but not at $p \leq 0.01$	
AMERICAN R. 89 VS AMERICAN R. 90	1
AMERICAN R. 90 VS BUMPING R. 90	3
BUMPING R. 89 VS NACHES R. 89	1
CLE ELUM 89 VS YAKIMA EASTON 89	3
YAKIMA EASTON 89 VS YAKIMA/EASTON 90	3
MARION DRAIN 89 VS MARION DRAIN 90	2
KLICKITAT H 90 VS KLICKITAT R. 90	1

Table 7. Stock contribution estimates (as percentage) for simulations of Yakima subbasin spring chinook mixed-stock samples. Each estimate is the mean of 50 simulations.

Simulation/Stocks	Stock Contributions 25-Fish Samples	50-Fish Samples	100-Fish Samples
Simulation: 100% American			
American	93 ± 9	95 ± 5	96 ± 4
Naches	6 ± 9	5 ± 5	4 ± 4
Yakima	1 ± 2	0 ± 1	0 ± 1
Simulation: 100% Naches			
American	11 ±12	10 ± 9	8 ± 7
Naches	81 ±16	83 ±13	87 ± 9
Yakima	8 ± 9	7 ± 7	5 ± 4
Simulation: 100% Yakima			
American	1 ± 2	0 ± 1	0 ± 1
Naches	6 ± 5	4 ± 4	4 ± 4
Yakima	93 ± 6	95 ± 5	96 ± 4

RESULTS AND DISCUSSION

Yakima Spring Chinook Substock Identification

Our approach to substock identification in the Yakima subbasin has been to sample virtually every sizable spawning aggregation, based on YIN spring chinook studies (Fast et al. 1988). Accordingly, in the Naches arm of the subbasin, the American River, Bumping River, Little Naches River, and mainstem Naches were sampled in both 1989 and 1990. In the Yakima arm, the Cle Elum River and mainstem Yakima at Easton were sampled in 1989. The 1989 data showed little genetic difference between fish sampled at the two locations, and with fish numbers being low, only the Yakima at Easton was sampled. In addition, a late spawning group downstream from Roza dam was sampled in both years. The peak of spawning was missed in 1989, resulting in only 14 fish, but a large sample was collected in 1990. We also collected a sample of the Carson hatchery stock, which had been released in the basin as late as 1986.

More existing information was available on Yakima spring chinook than on any other salmonid stock in the YKFP subbasins. The American River population had long been recognized as distinctive

because of its age structure (a high level of 5-year olds) and run timing (Howell et al. 1985). Howell et al. (1985) considered the upper Yakima population distinct from the American River stock because of differences in age structure and run timing, and considered the Naches spring chinook intermediate between the other stocks.

To a large extent, the expectations of substock structure based on the Howell et al. summary are borne out by the electrophoretic data. The two dendrograms (Figs. 1, 2) differ in some details, but both clearly show that the Yakima spring chinook collections cluster into two major groups: Naches and upper Yakima. However, the distinct life history characteristics of the American River population (Howell et al. 1985, and Knudsen, this report) are not strikingly reflected by the electrophoretic data. CSE chord distances separate the American River collections quite well from the other Naches collections (Fig. 2), but Nei distances (Fig. 1) demonstrate a close relationship between American River and Bumping River. The two American River collections differed overall in allele frequency (Table 6), the 1990 collection being more similar to Bumping River than the 1989. The inconsistency in the clustering of American River collections and similarity to the Bumping River collections may also reflect gene flow between American River and Bumping River. Gene flow is quite likely, since the American is a tributary of the Bumping, and both are relatively small streams. Considerable uncertainty still surrounds this situation because of the small sample sizes obtained thus far in Bumping River; relationships will be clarified as more data are collected.

The other major Howell et al.-based expectation of stock structure, that the Naches chinook are intermediate between upper Yakima and American River chinook, cannot be approached dendrogrammatically. Clustering in dendrograms is done agglomeratively, adding collections to existing clusters based only on which they are most similar to; relationships such as clines cannot be discerned from dendrograms (Lessa 1991). Intermediacy of the Naches population(s) has to be explored by locus-by-locus comparisons or by another ordination technique such as principal coordinate analysis (Gower 1966)(hereafter called PCOORD) or nonmetric multidimensional scaling (Lessa 1991).

PCOORD has been little used in fishery genetics research, but was used to advantage by Campton and Johnston (1985) in their study of Yakima rainbow trout. PCOORD presentations of Nei and CSE distances among the Yakima spring chinook collections are presented in Figs. 3 and 4, for purposes of comparison with the dendrograms. We regard our use of PCOORD at this point to be experimental, and caution against drawing firm conclusions from these diagrams. PCOORD presentations have obvious theoretical advantages over dendrograms in that the relationships are depicted in three dimensions rather than one, and there is no distortion of distances by agglomerative clustering. In actual application here, to a large extent they

mimic the dendrograms in depicting both the separation between Naches and upper Yakima, and the relationship between the American River and other Naches collections. The spatial placement of collections in Fig. 4 is clearly more plausible than that of Fig. 3 with respect to American River. In both figures, the 1990 American River collection (A2) is more similar to the other Naches collections than the 1989 collection (Al). In Fig. 4, it is closest to the Bumping River collections, and the Bumping collections are in turn somewhat separated from the other Naches collections; in Fig: 3, the 1990 American collection is closest to a Naches collection, and the Bumping collections are depicted as average Naches collections. On the issue of Naches intermediacy, the one area in which we hoped PCOORD may yield more information than dendrograms, the PCOORD plots agree, neither depicting the Naches as intermediate between the upper Yakima and American River.

PCOORD appears to provide a useful alternative to dendrograms in depicting genetic distances, and its use here again brings up a problem with genetic distance statistics we have noticed: often graphical presentations of CSE distances make more sense biologically than graphical presentations of the almost universally used Nei distances.

For prefacility purposes, it is necessary to partition these populations into substocks. To a large extent, however, substocks are management, rather than genetic, units in anadromous salmonids. While it would be convenient if the fish sorted themselves out into discrete, noninterbreeding units (true stocks), they naturally form a metapopulation, a group of partially isolated subpopulations. In other words, in trying to sort them into substocks we are to some extent fitting them into an unnatural classification scheme. What we have to do in dividing the populations into substocks for the YKFP is to make a decision as to what level of population differentiation is biologically meaningful. There are no universally applicable criteria for this, but we feel the recent NMFS Endangered Species Act species definition paper (Waples 1991) should be used as a guide. We propose that our criteria for describing YKFP substocks be at least as strict as'those used to describe "species" for ESA purposes. Thus, any YKFP population that could be considered a "species" sensu Waples (1991), should be considered a substock. For "species" status Waples requires that populations be evolutionarily significant units (ESU's), and for a population to be an ESU he requires that it: 1) be reproductively isolated (this need only be substantial, not total) from other populations, and 2) constitute an important component in the evolutionary legacy of the species. To meet the second requirement a population needs to be genetically distinct, to occupy a unique habitat, or to show evidence of unique adaptation to its environment. Using the ESA guidelines for substock identification is appealing for two reasons: 1) the guidelines provide a rational approach to dealing with a variety of data types, and 2) it will serve to minimize potential YKFP conflicts with the ESA. It is

important that the "at least as strict" aspect of the substock identification criteria be emphasized. One or more of the groups we may designate as substocks may not meet the criteria for ESU status, perhaps because of possible hatchery influence. This should in no way influence our treatment of these groups, because our charge in the YKFP is to protect the genetic integrity of the existing substocks, with no regard to significance outside the YKFP subbasins.

It seems clear from the genetic data, as summarized in the figures, that the upper Yakima and Naches populations should be regarded as separate substocks. Tagging data to verify reproductive isolation would be desirable, but even without it the genetic distances between the two groups relative to those within suggests that gene flow between the two arms of the subbasin is low. Within the upper Yakima there is no evidence of finer substock distinctions at this point. Although many of the possible G-tests are significant (Table 6), the Nei distances within the group are effectively zero. The CSE distances are much larger (Fig. 2), but the clustering of the two Yakima/Easton collections suggests that observed differences in the group may be due to sampling error. Should further data collection result in a situation where Yakima/Easton, Cle Elum, and Yakima below Roza collections tended to cluster by sampling location, the possibility of finer substock distinctions would have 'to be considered.

The situation is different in the Naches, because of the distinctiveness of the American River population and likely gene flow between it and the Bumping River population. An added complication is the heterogeneity between the American River collections, raising questions as to the "average" allele frequency profile of American River spring chinook and the "average" gene flow between American River and Bumping River (is it constant or highly variable?). The distinct life history characteristics and age distribution (Knudsen, this report) of American River is strong evidence for considering the American River population a substock distinct from the rest of the Naches. Among the other Naches collections there is little evidence for differentiation except for Bumping River, which is a link to American River. For the time being we consider the American River population and Naches (other than American) to be distinct substocks, but more data are needed. Except for the American River, sample sizes in the Naches have been fairly low. This is especially true of the Bumping, where much more accuracy in allele frequencies is needed. Similarly, data on gene flow within the Naches system, especially between the American and the rest of the basin, would be very useful.

Hatchery influence is to be expected in the Yakima, and the clustering of the Carson and upper Yakima stocks may reflect this, although we have no idea how similar the two were before the hatchery operations began. One CWT has been recovered in the collections made to date, a Leavenworth tag found in the Little

Naches in 1989 from a Cle Elum release. This is probably more noteworthy as an indicator of straying than as an indicator of hatchery influence.

Yakima Spring Chinook Mixed-Stock Fisherv Simulations

Simulations of mixed-stock Yakima spring chinook fisheries were carried out to do a preliminary evaluation of bias in our ability to monitor substock composition of a mixed group of fish, either in terminal fisheries, in broodstock collection, or perhaps in evaluation of winter migrants. The baseline used consisted of all 12 Yakima spring chinook collections, and fisheries consisting of 100% of each of the three stocks were simulated. All component collections of the stocks simulated were considered to exist in equal proportions in the fisheries. Fisheries of 25, 50, and 100 fish were simulated. For each run, 50 repetitions of the simulation were done. Results are presented in Table 7. In simulations of 100% American River, stock compositions averaged 95% American River and 5% Naches; there was essentially no allocation to the upper Yakima. The 100% Naches simulations allocated 81-87% to the Naches, with performance improving as sample size increased, and the remainder to American River and upper Yakima in an approximately 1.5:1 ratio. The upper Yakima simulations allocated 93-95% to upper Yakima with the remainder to the Naches; at most 1% was allocated to the American River. These simulations are preliminary, included only to provide a perspective on the problem. A much more comprehensive set of simulations, using mixed fisheries of the three stocks and differing mixtures of component collections within the stocks, needs to be done. However, in general it appears that American River and upper Yakima may be distinctive enough that their proportions can be estimated with relatively low bias. Estimation of Naches stock proportions involves considerably more bias.

Yakima Fall Chinook Substock Identification

The strategy for identification of fall chinook substocks in the Yakima subbasin differed markedly from that for spring chinook, because no evidence of substocks existed before our research began. In addition, poor visibility had hindered research to determine spawner distribution. It was believed, however, that distinctive substocks were unlikely to occur because of the large releases of hatchery upriver bright fall chinook into the basin in the last few years.

Besides the mainstem Yakima River, spawning was known to occur in Marion Drain, an irrigation channel west of the town of Granger. We decided initially to sample Marion Drain and the main stem Yakima (the most accessible site was Benton City). If no difference was found between these two collections, there would be little point in going further. Marion Drain was sampled in 1989 and in

1990, with sizable samples obtained in both years; an adequate sample from the mainstem Yakima was not collected until 1990.

Electrophoresis revealed that the mainstem and Marion Drain populations were quite distinctive (Figs. 1, 2). This distinctiveness is even more interesting when the allele frequency profiles of these collections are compared to other Columbia basin fall chinook. This was recently done in the course of preparing data for the ESA technical process (data not shown here). Two groups of upper Columbia fall chinook occur: one represented by Hanford Reach, Priest Rapids, and other "standard" upriver-bright hatchery stocks; and the other by Lyons Ferry hatchery (which produces Snake River fall chinook), Marion Drain, and possibly Deschutes River (only old, small samples are available). The mainstem Yakima collection clusters with the Hanford Reach/Priest Rapids group.

The similarity of the mainstem collection to this group is not surprising, given the large hatchery releases of recent years. In addition, five fish in the sample were coded-wire tagged, three from Priest Rapids releases in the Yakima and two from Priest Rapids releases in the Umatilla. The collective expansion of all five tags is 33 fish, mostly accounted for by the Umatilla fish, which each expanded to 12. In contrast, one Priest Rapids tag was recovered in Marion Drain in 1989 (and one clipped untagged fish), and none in 1990. If this pattern is typical, hatchery influence is stronger in the mainstem than in Marion Drain. This is to be expected, since all releases have been made into the mainstem.

The Marion Drain population probably represents original Yakima fall chinook, and the mainstem population represents original Yakima fall chinook overwhelmed by hatchery releases of Hanford Reach/Priest Rapids type fish. Releases having an impact on the mainstem group may include those outside the basin (such as Umatilla) as well as those within. It is also possible, however, that other native Yakima fall chinook substocks besides Marion Drain persist, and this possibility should be pursued.

There is speculation that the Marion Drain population resulted from colonization by exotic fall chinook, but no real evidence for this idea at this point. Howell et al. (1985) suggested the Marion Drain population was founded from a release of hatchery tules. This is refuted by the electrophoretic data; the Marion Drain fish are very distinct from tule stocks we have analyzed. In addition, Waples et al. (1991), based on the electrophoretic similarity of Lyons Ferry and Marion Drain fall chinook, suggest that Marion Drain may have been colonized by Snake fall chinook displaced by habitat destruction. This explanation for the Marion Drain-Lyons Ferry similarity is not supported by any other data. Marion Drain has been populated by fall chinook for many years, and genetically similar fish also occur in the Deschutes subbasin. The most likely cause of the Marion Drain-Lyons Ferry similarity is that both

populations (and the Deschutes fall chinook) represent the original genetically "typical" upriver fall chinook, and the Hanford Reach fall chinook, on which all the upriver bright fall chinook hatchery stocks are based, is genetically distinct.

One fact must be kept in mind in considering the future of the Marion Drain population: it accounts for a substantial portion of the genetic diversity existing among Columbia upriver fall chinook. Thus, it is a very important population in a basin-wide context.

Prosser Sprins and Fall Chinook Smolt Mixed Fishery Analysis

Two 100-fish collections of chinook smolts passing Prosser dam were made in early July 1990 to electrophoretically evaluate identifications of fish as spring or fall run. Collection W90DY consisted of fish over 90mm FL, collection W90DZ of fish under 90mm FL. Ninety fish from each collection were run through our MLE mixed-stock fishery program to estimate stock composition. The baseline used consisted of all the adult Yakima chinook collections. The stock composition of W90DY was estimated to be 60±6% falls; W90DZ was estimated to be 98±2% falls. At this time of year almost all smolts passing Prosser would be expected to be falls, so the results for W90DZ are within expectation. It also makes sense that the sample containing the larger fish would contain more spring chinook smolts. The breakdown of allocations by substock is interesting, although it should be considered tentative because of potential bias problems (MLE estimates are biased- the more similar the baseline stocks, the more serious the potential bias). The fall chinook contribution in W90DY was all from the main stem, and the spring chinook contribution all from the upper Yakima (Cle Elum, Easton, and Below Roza). The fall chinook contribution in W90DZ was 79% mainstem and 18% Marion Drain, and the spring chinook contribution all from the Naches group (Naches, Little Naches, and Bumping).

Klickitat Spring Chinook Substock Identification

Little is known about the historical distribution and abundance of wild Klickitat spring chinook, large-scale hatchery releases have been made into the subbasin since the late 1950s from a WDF hatchery sited 40 miles upstream from the Columbia confluence. Thus there was no existing evidence for substock structure, and a high likelihood that, had there been multiple substocks, they would have been overwhelmed by the hatchery releases. Accordingly, our research has been aimed at one central question: are there spring chinook in the Klickitat that are genetically distinct from the hatchery stock?

The Klickitat hatchery stock was sampled in both 1989 and 1990. In addition, Carson fish returning to Klickitat in 1990 were sampled.

These were the product of a large Carson release in 1986 designed to meet an **eggtake** shortfall of the Klickitat hatchery stock. The Carson fish were all ventrally clipped, and were supposed to be spawned separately from the returning Klickitat fish, but some crossbreeding was observed by our sampling staff. This crossbreeding, if substantial, will cause a shift in the Klickitat allele frequency profile in the 1990 brood year and the next few brood years descended from it, as the Carson and Klickitat stocks differ substantially in allele frequency (Tables 3, 6; Figs. 1, 2). The Carson fish are no longer on-station, so additional crossbreeding will not occur in future years. The largest sample of wild spawners obtained to date was 35 fish in 1990.

The wild spawners do appear distinct from the hatchery stock (Figs. 1, 2; Table 6). This may in part be attributed to aberrant allele frequencies caused by the small sample size, but not entirely. The wild collection included two heterozygotes for the sAAT-1,2*105 allele, which is a fairly rare allele found in neither the collections of the Klickitat hatchery stock nor the Carson samples (Carson and Carson at Klickitat). This is an uncommon but widely distributed allele that is known to occur in the Fraser drainage (D. Teel, NMFS, **pers.** comm.j and in coastal California (Gall et al. 1989). In the Columbia basin, outside of this occurrence in the Klickitat subbasin, it is known to occur only in the Wenatchee subbasin. There is a possibility of mistaking degradation products for this allele on the gels, so the samples were rerun. The variation appears to be real.

The finding of rare variation in the collection of wild spawners is surprising, based on presampling expectations, but even more so given that at least six hatchery fish (identified by scale patterns) were included in the collection. These six fish included neither of the rare heterozygotes.

Further sampling is obviously warranted, but at this point the evidence indicates that there is a group of Klickitat natural spawners that are genetically distinct from the hatchery stock.

SUMMARY RECOMMENDATIONS

Yakima Spring Chinook

Although a great deal of electrophoretic data has been accumulated on YKFP spring chinook stocks, particularly those in the Yakima, it is important to keep in mind two limitations of the data. First, an electrophoretic profile of the stocks gives us some insight into the substock structure, but only at that moment in time. Each year of sampling is essentially a snapshot of a dynamic process. By sampling repetitively and examining allele frequency shifts we can begin to see the dynamic process. For this reason we originally proposed, and plan to continue prefacility sampling through one

complete generation. Even this extensive sampling, however, is insufficient to give us as clear a picture of genetic relationships among Yakima spring chinook as we need for careful management of the substocks. Tagging studies should be done to evaluate gene flow within and among the substocks. This should be complemented by computer simulations to explore how the relationships among the substocks will change under various gene flow regimes.

The second limitation is precision of data. All these data are subject to sampling error that is dependent on sample size. If allele frequencies in two collections differ, no matter what the sample size, this will be reflected in any graphical representation of genetic relationships. Much of the resulting pattern of relationships may be spurious, however, based largely on sampling error. At this point there is a large disparity in sample sizes of the Yakima spring chinook collections; American River and upper Yakima collections tend to be large, but Naches collections are substantially smaller (especially Bumping River). Repeat sampling of populations will remove much of the uncertainty about allele frequency profiles, but if possible, larger Naches collections need to be made.

Alternative methods of population ordination, such as principal coordinates and multidimensional scaling, should be further developed to better define relationships among the substocks. More work should be done to find a "best" genetic distance statistic; a logical starting point is a comparison of Hillis' (1984) modified Nei distance with the orthodox Nei and CSE distance statistics.

Simulation analysis should be expanded to potentially aid in monitoring substock composition in terminal fisheries, in broodstock collection, as well as other uses such as evaluation of winter migrants. This will be especially important for harvest strategies to minimize impacts on American River, which is to be a genetic refuge stock, but will also be useful in evaluating the substock composition of winter migrants.

Yakima Fall Chinook

Sampling of Marion Drain and the mainstem should be continued, and sampling efforts should be expanded to explore the possibility of Marion Drain-type fish existing elsewhere in the subbasin. The hypothesis that the mainstem fish genetically represent an admixture of Marion Drain-type fish and hatchery upriver brights should be tested. The Deschutes River fall chinook population should be sampled to better delineate its relationship to the Marion Drain and Lyons Ferry populations.

Klickitat Spring Chinook

The present sampling program should be continued, emphasizing efforts to collect a larger sample of natural spawners. Sampling should be expanded to any other areas in the subbasin where the existence of additional substocks is plausible. The tribal dipnet fishery should be sampled for wild fish. An estimate of the numerical importance of wild fish to the overall run can be estimated using scale pattern analysis, and those fish identified as wild can be analyzed electrophoretically for comparison with the Klickitat spring chinook samples already collected.

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Appendix 1. 1991 WDF chinook electrophoretic baseline protocol

HEART

TRIG-GLY (35mm origin) 5 1/4 hrs @ 600V (max. 90 mA) LKB THICK GEL
- PEPB (PEPB-1 & 2)
- PGM (PGM-1, 2) score quickly
- HAGH (HAGH + ACR)
- MPI
- ADA (ADA-l & 2)
- SOD (sSOD-1 & mSOD) a + c from middle

CAMEN 6.8 (35mm origin) 5 1/4 hrs @ 250V (max. 90 mA) THICK GEL
ADD 15mg NAD/100mL gel buffer immediately before degassing
- G3PDH (G3PDH-3)
- AH (mAH-1, 2, 3, & 4)
- MDH (sMDH-A1,2 & B1,2 & mMDH-1, 2, & 3) a + c
- AAT (mAAT-1) c only from middle
- IDHP + PGDH (mIDHP-1 & 2 + PGDH) score IDH very quickly
- PEPD (PEPD-2)
- GAPDH (GAPDH-2 & 3)
- MEP (mMEP) add 15 mg oxaloacetate to stain

TC-4 (40mm origin) 5 hrs @ 90 mA (max. 250V) LKB THICK GEL
[usk ofHeathkit may reguire longer run]
- PEPB (PEPB-1) a + c
- AAT (sAAT-1,2 & mAAT-1 & 2) a + c
- MEP (sMEP-1 & 2) score quickly use 15 mg. oxaloacetate
- SOD (sSOD-1 & 2 & mSOD) a + c
- GR
- IDHP (sIDHP-1,2)

Appendix 1. (cont.)

EYE

TRIS-GLY (35mm origin) 5 1/4 hrs @ 550V (max. 80 mA) LKB THIN GEL
LDH (LDH-Bl, B2, & C)
AAT (sAAT-3)
TPI (TPI-1.1, 1.2, 2.1 & 2.2)
PEPA (PEPA) score quickly
HAGH

CAME 6.8 (35mm origin) 5 1/4 hrs @ 250V (max. 75 mA) THIN GEL
AAT (sAAT-3) (200 mg fast blue BB)
IDHP (sIDHP-1,2)
PGK (PGK-2) score quickly
GR
LDH (LDH-Bl, B2, & C)

[MDH instead of GR?]

MUSCLE

TRIS-GLY (35 mm origin) 5 1/4 hrs @600V (max. 90 mA) LKB THICK GEL
PEPB (PEPB-1)
PGM + MPI (PGM-1 & 2) score PGM quickly
GPI (GPI-B1, B2, A & r) score very quickly
PEP-LT (PEPD-2 & PEP-LT)
TPI (TPI-1.1, 1.2, 2.1, & 2.2) a + c
ADA (ADA-l & 2)
CK (CK-Al & A2)

CAME 6.8 (35mm Origin) 5 1/4 hrs @ 250V (max. 90 mA) THICK GEL
AH (mAH-3 & 4)
PGK (PGK-2) score quickly
MDH (sMDH-A1,2 & B1,2 & mMDH-2, & 3) a + c
AAT (sAAT-1,2 & mAAT-1) a + c
IDHP + PGDH (mIDHP-1, 2 & sIDHP-1, & 2 + PGDH)
G3PDH (G3PDH-4)

TC-4 (40mm origin) 5 hrs @ 90 mA (max. 250V) LKB THICK GEL
[use of Heathkit may reguire longer run]
PEP-LT + PEPB (PEP-LT + PEPB-1) a + c
AAT (mAAT-1 & 2) c Only, from middle
IDHP (sIDHP-1, & 2) esp. "94" allele
MEP (sMEP-1 & 2) use 15mg oxaloacetate
GR
PEPD (PEPD-2)
ADA (ADA-2)

Appendix 1. (cont.)

LIVER

CAME 6.8 (35 mm origin) 5 1/4 hrs @ 250V (max. 80 mA) THIN GEL
- LDH (LDH-B2)
- AAT (sAAT-4)
- AH (sAH)
- IDHP (sIDHP-1, & 2)

[try GR on extra slice]

LIOH-RW (45mm origin) 80 mA (max. 400V) LRB THIN GEL
run until buffer front is 1 cm from end of gel
- IDDH (IDDH-1 & 2) a + c
- AAT (sAAT-4)
- AH (sAH)
- SOD (sSOD-1) a + c

Appendix 2. Chinook variable loci and alleles.

WDF Allele Codes & Standard Relative Mobilities

LOCUS	A	B	C	D	E	F	G	H	I	J	TISSUE
sAAT-1,2	100	85	105	(91[a])							M,H
sAAT-3	100	90	113	95'	71[a]						E
sAAT-4	100	130	63								L
mAAT-1	-100	-77	-104	XX(-119)[a]							M,H
mAAT-2[b]	-100	[-125]	[-90]								M,H
mAAT-3[b]	loo-	-450									H
ADA-l	100	83	(69')	96[a]	f[a]						M,E,H
ADA-2	100	105	96'								M,E,H
sAH	100	86	112[c]	108[c]	69	118'					L
mAH-1	100	65									H
mAH-2[b]	100	88									H,E
mAH-3	100	126	74								M,H
mAH-4	100	119	112	109[a]	(136[a])						M,H
CK-A1[b]	100	-450									M
CK-C2[b]	100	[105]	[95]								E
CK-B[b]	100	96									E
GAPDH-2[b]	100	22									H
GAPDH-3[b]	100	123									M,H
bGA[b]	100	60									L
GPI-A	100	105	93	85'							M,E,H
GPI-B1[b]	100	xx	(175)								M
GPI-B2	100	60	135	24							M
GPIr	100	%									M
GR	100	85	110	89[a]	mf[a]	71[a]	(115[a])				M,E,H
G3PDH-3[b]	100	112									H
HAGH	100	143	131[a]	65[a]	28[a]						M,H,L
IDDH-l[b]	100	0									L
IDDH-2[b]	100	61									L
mIDHP-1[b]	100	147	(30)	178							M,E
mIDHP-2	100	154	50'								M,E
sIDHP-1,2	100	127	74	142	50	94	(83)	129	136[a]	92[a] &&	M,E,L
sIDHP-1	100		74	142	94		(83)	129	136[a]	92" &&	M
sIDHP-2	100	127		142	50		(83)			&&	
LDH-B1[b]	100	(-60)									E,L
LDH-B2	100	112	134	71	(56[a])						E,L
LDH-C	100	90	84								E
sMDH-A1,2	100	120	27	-45	(160')						M,H,E
sMDH-B1,2	100	121	70	83	126'	0/f[a]	0/s[a]				M,H,L
mMDH-1	-100	-900									M,H
mMDH-2	100	200	-180'								M,H
sMEP-1	100	92	105	86'							M,H
sMEP-2	100	{78}									M,H
mMEP[b]	100	-75									H
MPI	100	109	95	113	103'	ms[a]	vs[a]				M,H,E
PEPA	100	90	86	81[a]	XX(~111[a])						M,E,H
PEPB-1	100	130	-350	(s' = old 45 or 68 ?)							M,H,E,L
PEPB-2	100	108									M,H
PEPD-2	100	107	83[a]								M,H
PEP-LT	100	110	(120')	88[a]							M,H
PGDH	100	90	85	(95[a])	(109[a])						M,E,H

Appendix 2.' (cont.)

WDF Allele Codes & Standard Relative Mobilities

LOCUS	A	B	C	D	E	F	G	H	I	J	TISSUE
PGK-2	100	90	74'	(ms[a])							M,E,L
PGM-1	100	210	165'	50[a]							M,H
PGM-2	100	166	136(~145[a])	63'							M,H,L
PGM-3,4[b]	100	96	90	108	86						H,L
sSOD-1	-100	-260	580	1260	-175[a](-160[a])						M,H,E
sSOD-2[b]	100	[120]									H
mSOD-1	100	142	141[a,d]								M,H
TPI-1.1[b]	-100(-121?)										M,E
TPI-1.2[b]	-100	-400									M,E
TPI-2.1[b]	100	[104]	[106]	[91]	[96]						H,E,M
TPI-2.2	100	[104]	[75[a]]	[96[a]]	[102[a]]	[101[a]]					M,E

[a] = allele is not currently recognized in the coast-wide baseline
[b] = locus is not currently supported by the coast-wide baseline
[c] = mobility standards are necessary to distinguish the *108 and *112
[d] = allele has approximately the same mobility as the *142 (on EBT and LIOH-RW(E@) but not on TC-4) and has greatly reduced activity, therefore the phenotypes are distinguishable (this may actually be simply an artifact; it has not been observed since 1986!)
( ) = allele has only been seen in mixed-stock fishery samples
[ ] = scoring of variant & mobility of allele determined from interlocus heterodimeric isozymes
{ } = allele does not generate an isozyme of different mobility and is only scored reliably in the homozygous state
% = allele represents the absence of the GPI A/B1 heterodimer
XX = this allele code not presently used
&& = the *K allele is *66 and is from sIDHP-2; the *L allele is coded as *126 and is from sIDHP-1; the *M allele is *72 (TC-4) and is from sIDHP-1; the *N allele is approximately *132 and is probably from sIDHP-1.

GENETIC ANALYSIS OF YAKIMA RIVER STEELHEAD: INITIAL ANALYSIS OF WITHIN BASIN GENETIC DIVERSITY AND COMPARISON TO HATCHERY STEELHEAD AND RAINBOW TROUT.

PURPOSE

The primary purpose of this work was to electrophoretically characterize steelhead collections from the Yakima River and tributaries to determine the stock structure. An additional purpose was to compare Yakima steelhead to hatchery steelhead and rainbow trout strains to estimate the amount of gene flow from nonnative gene pools.

METHODS

Wild-spawned steelhead smolts were collected during outmigration in 1989 and 1990 from six locations in the Yakima River (Table 8). Adult broodstock were sampled from the 1990 hatchery spawning at the Yakima Hatchery and smolts were collected from Nile Pond. Four Washington Department of Wildlife (WDW) hatchery rainbow trout strains were also sampled (work on wild Yakima rainbow trout is in progress). Stocked hatchery-origin steelhead were identified by an adipose-fin clip and excluded from the collections. The collected fish were frozen at ultra-low temperatures (-80°C) and transported to the Washington Department of Fisheries (WDF) Genetic Stock Identification Laboratory. Unfortunately, some of the smolts collected in 1990 thawed prior to electrophoresis, which resulted in poor sample quality and loss of enzyme activity at several loci.

We split the 1989 Prosser smolt collection into four components based on outmigration timing to test for the presence of multiple steelhead stocks in the Yakima Basin (Prosser 89-l May 3-11, Prosser 89-2 May 14-18, Prosser 89-3 May 22-30, Prosser 89-4 June 2-14). We tested for significant genotypic frequency deviations from expected Hardy-Weinberg equilibrium, heterogeneity in allele frequencies, and examined the component collections for gametic disequilibrium.

Muscle, heart, eye and liver were dissected from each smolt and placed into 12 X 75 mm test tubes. Total length, weight, and 12 scales from the preferred area were taken. The smolts were photographed and refrozen for storage.

Electrophoresis followed the methods of Aebersold et al. (1987). The electrophoretic protocol, enzymes screened, and alleles observed during this study (and other studies on rainbow trout and steelhead by WDF) are listed in Appendices 3, 4, and 5. Genetic nomenclature follows the conventions of Shaklee et al. (1990).

BIOSYS-1 (Swofford and Selander 1981) was used for the statistical analysis of the electrophoretic data. The gametic disequilibrium program was written by Dr. Peter Smouse and adapted to the WDF computer by Craig Busack.

Table 8. Steelhead collections from the Yakima River and tributaries during 1989 and 1990, and WDW rainbow trout strains.

Category	Location	Year/Subsample	Sample Size
Wild Steelhead			
	Dry Cr.	1989	84
	Logy Cr.	1990	77
	Satus Cr.	1990	98
	Wapatox	1989	158
	Wapatox	1990	100
	Roza	1989	54
	Prosser	1989-1	77
	Prosser	1989-2	60
	Prosser	1989-3	87
	Prosser	1989-4	48
Hatchery Steelhead			
	Yakima	1990	49
	Nile Pond	1990	50
Hatchery Rainbow			
	Goldendale	1990	100
	Spokane	1990	100
	Tokul	1990	100
	S. Tacoma	1990	100
	Naches (Goldendale strain)	1990	53

RESULTS

We resolved the products of 59 loci and identified genetic variation at 42 loci during the analysis of the Yakima steelhead and WDW hatchery collections (duplicate isoloci are counted as two loci)(Table 9a-b). The average heterozygosity and percentage of polymorphic loci were typical for steelhead (Table 10).

Table 9a. Allele frequencies in collections 1 through 9

Locus	Collection 1	2	3	4	5	6	7	8	9
sAAT-1,2									
(N)	84	77	98	49	158	100	50	54	77
A	0.988	0.990	0.997	0.990	0.990	1.000	0.995	0.986	0.990
B	0.012	0.009	0.003	0.010	0.007	0.000	0.000	0.009	0.007
C	0.000	0.000	0.000	0.000	0.003	0.000	0.005	0.005	0.003
sAAT-3									
(N)	84	78	102	43	159	100	50	54	77
A	0.982	1.000	0.975	0.942	1.000	0.995	1.000	0.991	0.987
B	0.018	0.000	0.025	0.058	0.000	0.005	0.000	0.009	0.013
mAAT-1									
(N)	84	78	102	48	117	98	49	54	67
A	1.000	0.974	0.961	0.979	0.987	1.000	0.969	0.991	0.978
B	0.000	0.026	0.039	0.021	0.013	0.000	0.031	0.009	0.022
ADA-l									
(N)	84	78	102	48	159	96	49	54	77
A	0.988	0.994	0.995	0.979	0.943	0.958	0.867	0.898	0.935
B	0.012	0.006	0.005	0.021	0.057	0.042	0.133	0.102	0.065
ADA-2									
(N)	84	78	100	49	159	76	15	54	77
A	0.994	0.974	0.950	0.980	0.987	1.000	1.000	0.981	0.994
B	0.006	0.026	0.050	0.020	0.003	0.000	0.000	0.000	0.006
C	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.019	0.000
ADH									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	0.994	1.000	1.000	0.997	1.000	1.000	1.000	1.000
B	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
sAH									
(N)	84	78	98	49	159	97	46	54	77
A	0.726	0.737	0.745	0.714	0.824	0.814	0.750	0.806	0.818
B	0.274	0.237	0.255	0.286	0.176	0.186	0.250	0.194	0.182
C	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000
mAH-1									
(N)	84	78	28	49	159	77	12	54	77
A	1.000	1.000	1.000	0.990	0.975	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.010	0.025	0.000	0.000	0.000	0.000
mAH-2									
(N)	84	78	57	49	159	77	15	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
mAH-3									
(N)	84	78	102	49	159	77	15	54	77
A	1.000	1.000	1.000	0.990	0.997	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.010	0.003	0.000	0.000	0.000	0.000

Table 9a (cont.)

LOCUS	Collection 1	2	3	4	5	6	7	8	9
mAH-4									
(N)	84	78	57	49	159	77	15	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AK									
(N)	84	78	102	31	153	100	50	54	65
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ALAT									
(N)	84	78	101	47	159	**99**	44	54	76
A	0.857	0.872	0.896	0.840	0.912	**0.929**	0.989	0.935	0.895
B	0.143	0.128	0.104	0.149	0.069	0.061	0.011	0.056	0.099
C	0.000	0.000	0.000	0.000	0.013	0.010	0.000	0.000	0.007
D	0.000	0.000	0.000	0.011	0.006	0.000	0.000	0.009	0.000
CK-Al									
(N)	84	78	102	48	159	100	50	54	77
A	1.000	1.000	1.000	0.990	1.000	1.000	1.000	0.991	1.000
B	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.009	0.000
CK-A2									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	0.985	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000
CK-B									
(N)	84	78	102	46	159	100	50	**54**	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**	1.000
CK-Cl									
(N)	84	18	102	**32**	159	93	50	**54**	**67**
A	1.000	1.000	1.000	**1.000**	0.997	0.989	0.990	**0.991**	**0.985**
B	0.000	0.000	0.000	0.000	0.003	0.011	0.010	0.009	0.015
CK-C2									
(N)	84	18	102	34	159	77	50	54	67
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-3									
(N)	30	78	102	16	96	100	50	42	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-4									
(N)	30	78	102	34	96	100	**50**	**42**	**77**
A	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**	**1.000**	**1.000**
GR									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-Bl									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.991	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000

Table 9a (cont.)

	Collection								
Locus	1	2	3	4	5	6	7	8	9
GPI-B2									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
GPI-A									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	0.987	0.985	1.000	1.000	0.994
B	0.000	0.000	0.000	0.000	0.009	0.005	0.000	0.000	0.006
C	0.000	0.000	0.000	0.000	0.003	0.010	0.000	0.000	0.000
G3PDH-1									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	0.997	0.995	0.990	1.000	1.000
B	0.000	0.000	0.000	0.000	0.003	0.005	0.010	0.000	0.000
G3PDH-2									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
HAGH									
(N)	84	78	102	**49**	159	100	**50**	**54**	**77**
A	1.000	1.000	1.000	**1.000**	1.000	1.000	**1.000**	**1.000**	**1.000**
IDDH-1									
(N)	84	78	95	30	111	96	47	54	55
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
IDDH-2									
(N)	84	78	96	30	153	96	47	54	65
A	0.988	0.994	1.000	0.983	0.984	1.000	1.000	0.991	0.992
B	0.012	0.006	0.000	0.017	0.000	0.000	0.000	0.009	0.008
C	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
mIDHP-1									
(N)	84	**77**	94	**49**	159	**99**	50	54	77
A	1.000	**1.000**	1.000	**1.000**	0.994	**0.995**	1.000	0.981	1.000
B	0.000	0.000	0.000	0.000	0.006	0.005	0.000	0.019	0.000
mIDHP-2									
(N)	84	78	102	49	159	100	50	54	76
A	0.988	0.994	0.980	0.969	0.950	0.990	0.990	0.889	0.934
B	0.012	0.006	0.020	0.031	0.047	0.010	0.010	0.111	0.066
C	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000

Table 9a (cont.)

Locus	Collection 1	2	3	4	5	6	7	8	9
sIDHP-1,2									
(N)	84	78	101	49	159	100	50	54	77
A	0.550	0.561	0.576	0.536	0.599	0.570	0.565	0.680	0.614
B	0.223	0.180	0.228	0.260	0.223	0.220	0.245	0.162	0.214
C	0.000	0.003	0.008	0.000	0.009	0.003	0.000	0.000	0.007
D	0.193	0.211	0.174	0.178	0.152	0.200	0.185	0.129	0.159
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.006	0.003	0.010	0.000	0.003	0.000	0.000	0.005	0.000
G	0.015	0.038	0.005	0.020	0.009	0.008	0.005	0.018	0.003
H	0.009	0.003	0.000	0.005	0.002	0.000	0.000	0.005	0.003
I	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
K	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
LDH-Al									
(N)	84	78	102	48	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.991	0.994
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.006
LDH-A2									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-B1									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-B2									
(N)	84	78	102	49	159	100	50	54	77
A	0.554	0.526	0.637	0.653	0.519	0.515	0.590	0.602	0.623
B	0.440	0.474	0.358	0.347	0.481	0.485	0.400	0.398	0.377
C	0.006	0.000	0.005	0.000	0.000	0.000	0.010	0.000	0.000
LDH-C									
(N)	84	78	102	48	159	100	50	54	77
A	1.000	1.000	1.000	0.979	0.997	0.995	1.000	0.981	0.981
B	0.000	0.000	0.000	0.021	0.003	0.005	0.000	0.019	0.019
sMDH-A1,2									
(N)	84	78	102	49	159	100	50	54	77
A	0.997	0.993	0.997	0.995	0.997	0.995	1.000	0.995	0.990
B	0.003	0.007	0.003	0.005	0.003	0.005	0.000	0.005	0.007
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
sMDH-B1,2									
(N)	84	77	102	49	159	100	49	53	77
A	0.994	0.993	0.990	0.974	0.984	0.992	0.980	0.944	0.968
B	0.003	0.000	0.000	0.010	0.002	0.000	0.005	0.000	0.003
C	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.009
D	0.003	0.000	0.000	0.015	0.011	0.008	0.010	0.033	0.016
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.000
F	0.000	0.013	0.020	0.000	0.000	0.000	0.010	0.000	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000

Table 9a (cont.)

Locus	Collection 1	2	3	4	5	6	7	8	9
mMDH-2									
(N)	84	74	74	49	**159**	**99**	37	54	77
A	1.000	1.000	1.000	1.000	**0.987**	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
mMDH-3									
(N)	84	78	68	49	159	99	37	54	76
A	1.000	1.000	1.000	1.000	0.997	0.990	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.003	0.010	0.000	0.000	0.000
mMEP-1									
(N)	84	78	102	49	159	33	15	54	67
A	0.976	1.000	1.000	1.000	0.991	1.000	1.000	1.000	0.985
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.024	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.015
MPI									
(N)	84	78	102	46	159	100	50	54	77
A	0.899	0.865	0.907	0.826	0.928	0.895	0.870	0.907	0.870
B	0.095	0.115	0.093	0.174	0.069	0.105	0.130	0.093	0.130
C	0.006	0.019	0.000	0.000	0.003	0.000	0.000	0.000	0.000
PEP-LT									
(N)	84	61	1	49	159	24	41	54	**77**
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEPA									
(N)	84	57	100	47	159	70	6	54	75
A	0.804	0.930	0.835	0.851	0.937	0.986	1.000	0.815	0.887
B	0.196	0.070	0.165	0.149	0.063	0.014	0.000	0.167	0.113
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
PEPD									
(N)	84	78	30	49	159	61	50	54.	77
A	0.988	1.000	1.000	1.000	1.000	0.967	1.000	1.000	0.987
B	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006
C	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.006
PGDH									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	**1.000**	1.000	1.000	1.000	1.000	1.000	1.000
PGK-2									
(N)	84	78	102	48	159	100	44	54	77
A	0.571	0.590	0.569	0.542	0.497	0.465	0.375	0.574	0.623
B	0.423	0.410	0.422	0.427	0.472	0.480	0.614	0.389	0.344
C	0.006	0.000	0.010	0.021	0.028	0.035	0.000	0.037	0.026
D	0.000	0.000	0.000	0.010	0.003	0.020	0.011	0.000	0.006

Table **9a** (cont.)

	Collection								
Locus	1	2	3	4	5	6	7	8	9
PGM-2									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	0.978	0.985	0.950	0.981	0.987
B	0.000	0.000	0.000	0.000	0.016	0.015	0.050	0.019	0.006
C	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.006
PNP									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
sSOD-1									
(N)	84	78	102	49	159	92	50	54	76
A	0.964	0.833	0.877	0.857	0.808	0.815	0.850	0.935	0.928
B	0.006	0.006	0.020	0.031	0.085	0.060	0.030	0.028	0.020
C	0.030	0.160	0.103	0.112	0.107	0.125	0.120	0.037	0.053
sSOD-2									
(N)	55	18	75	49	149	1	10	13	23
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-1									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-2									
(N)	84	78	102	49	159	100	50	54	77
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-4									
(N)	84	71	102	49	159	76	15	54	77
A	0.899	0.915	0.824	0.867	0.981	0.961	0.933	0.944	0.955
B	0.101	0.085	0.176	0.133	0.019	0.039	0.067	0.056	0.045

Table 9b. Allele frequencies'in populations 10 through 17

	Collection							
LOCUS	10	11	12	13	14	15	16	17
sAAT-1,2								
(N)	60	87	48	100	100	100	100	53
A	0.991	0.997	0.995	1.000	1.000	1.000	1.000	1.000
B	0.008	0.003	0.005	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
sAAT-3								
(N)	60	87	48	100	100	100	100	53
A	0.983	0.994	0.990	1.000	1.000	1.000	1.000	1.000
B	0.017	0.006	0.010	0.000	0.000	0.000	0.000	0.000
mAAT-1								
(N)	60	87	26	98	100	100	100	53
A	0.983	0.994	0.981	0.990	0.960	1.000	0.920	1.000
B	0.017	0.006	0.019	0.010	0.040	0.000	0.080	0.000
ADA-l								
(N)	60	87	48	68	30	80	95	53
A	0.850	0.914	0.865	0.206	0.500	0.344	0.158	0.236
B	0.150	0.086	0.135	0.794	0.500	0.656	0.842	0.764
ADA-2			.					
(N)	60	87	48	100	100	100	100	37
A	0.983	0.983	0.990	1.000	1.000	1.000	1.000	1.000
B	0.017	0.006	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.011	0.010	0.000	0.000	0.000	0.000	0.000
ADH								
(N)	60	86	48	100	100	100	100	53
A	1.000	1.000	0.990	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
SAH								
(N)	60	87	48	100	100	100	100	53
A	0.833	0.793	0.833	1.000	1.000	1.000	0.905	1.000
B	0.158	0.201	0.167	0.000	0.000	0.000	0.095	0.000
C	0.008	0.006	0.000	0.000	0.000	0.000	0.000	0.000
mAH-1								
(N)	60	87	48	100	98	100	100	29
A	1.000	1.000	0.979	1.000	0.980	1.000	1.000	1.000
B	0.000	0.000	0.021	0.000	0.020	0.000	0.000	0.000
mAH-2								
(N)	59	87	48	100	100	100	100	37
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
mAH-3								
(N)	59	87	48	100	100	100	100	37
A	1.000	1.000	1.000	0.995	1.000	0.965	1.000	1.000
B	0.000	0.000	0.000	0.005	0.000	0.035	0.000	0.000

Table 9b (cont.)

	Collection							
Locus	10	11	12	13	14	15	16	17
nlAH-4								
(N)	59	87	48	100	100	100	100	37
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AK								
(N)	23	87	36	65	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ALAT								
(N)	60	87	48	100	100	100	100	53
A	0.933	0.920	0.927	1.000	1.000	0.990	1.000	1.000
B	0.058	0.080	0.062	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.010	0.000	0.000	0.010	0.000	0.000
D	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CK-Al								
(N)	60	87	48	99	99	100	100	53
A	0.950	1.000	0.979	0.939	0.889	0.750	1.000	0.953
B	0.050	0.000	0.021	0.061	0.111	0.250	0.000	0.047
CK-A2								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CK-B								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-Cl								
(N)	41	**29**	48	100	100	100	97	53
A	1.000	**0.983**	0.969	1.000	1.000	1.000	0.686	1.000
B	0.000	0.017	0.031	0.000	0.000	0.000	0.314	0.000
CK-C2								
(N)	41	29	48	100	100	100	100	47
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-3								
(N)	57	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH-4								
(N)	57	87	48	100	100	100	99	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GR								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-Bl								
(N)	60	87	48	100	100	100	100	53
A	1.000	0.989	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000

Table 9b (cont.)

	Collection							
Locus	10	11	12	13	14	15	16	17
GPI-B2								
(N)	60	87	48	100	100	100	100	53
A	1.000	0.994	1.000	1.000	1.000	1.000	1.000	0.981
B	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.019
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GPI-A								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	0.979	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
G3PDH-1								
(N)	60	87	48	100	100	100	100	53
A	0.992	1.000	0.979	1.000	0.960	1.000	1.000	1.000
B	0.008	0.000	0.021	0.000	0.040	0.000	0.000	0.000
G3PDH-2								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
H A G H								
(N)	60	87	47	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDDH-1								
(N)	23	87	14	99	100	100	100	29
A	1.000	0.994	1.000	0.763	0.945	1.000	1.000	0.862
B	0.000	0.000	0.000	0.237	0.055	0.000	0.000	0.121
C	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.017
IDDH-2								
(N)	23	87	36	100	100	100	100	47
A	0.978	0.983	0.972	0.925	1.000	1.000	1.000	1.000
B	0.022	0.017	0.028	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.075	0.000	0.000	0.000	0.000
mIDHP-1								
(N)	60	87	48	100	100	100	100	53
A	0.992	1.000	0.990	1.000	1.000	1.000	1.000	1.000
B	0.008	0.000	0.010	0.000	0.000	0.000	0.000	0.000
mIDHP-2								
(N)	60	87	48	98	100	100	100	53
A	0.900	0.948	0.865	0.628	0.870	0.360	0.765	0.651
B	0.100	0.052	0.135	0.372	0.130	0.640	0.235	0.349
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 9b (cont.)

	Collection							
LOCUS	10	11	12	13	14	15	16	17
sIDHP-1,2								
(N)	60	87	48	100	100	91	65	52
A	0.629	0.597	0.630	0.847	0.512	0.805	0.523	0.856
B	0.171	0.198	0.198	0.127	0.327	0.063	0.323	0.120
C	0.000	0.008	0.000	0.000	0.020	0.000	0.031	0.000
D	0.179	0.178	0.136	0.025	0.042	0.132	0.123	0.024
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.003	0.005	0.000	0.098	0.000	0.000	0.000
G	0.016	0.011	0.026	0.000	0.000	0.000	0.000	0.000
H	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
K	0.000	0.003	0.005	0.000	0.000	0.000	0.000	0.000
LDH-Al								
(N)	60	87	48	99	99	100	100	53
A	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000
LDH-A2								
(N)	60	87	48	99	99	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-B1								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-B2								
(N)	60	87	48	100	100	100	100	53
A	0.558	0.621	0.687	1.000	1.000	1.000	1.000	1.000
B	0.442	0.379	0.312	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-C								
(N)	60	87	48	100	100	100	100	53
A	0.958	0.977	0.969	0.900	0.840	0.750	0.820	0.953
B	0.042	0.023	0.031	0.100	0.160	0.250	0.180	0.047
sMDH-A1,2								
(N)	60	87	48	100	100	100	100	53
A	1.000	0.994	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
sMDH-B1,2								
(N)	60	87	48	100	100	100	100	53
A	0.925	0.977	0.974	0.710	0.805	0.802	0.862	0.717
B	0.004	0.003	0.000	0.012	0.000	0.000	0.000	0.000
C	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000
D	0.054	0.008	0.021	0.277	0.195	0.197	0.137	0.278
E	0.025	0.011	0.010	0.000	0.000	0.000	0.000	0.000
F	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000
G	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009

Table 9b (cont.)

	Collection							
Locus	10	11	12	13	14	15	16	17
mMDH-2								
(N)	60	87	48	99	96	100	100	39
A	0.992	0.989	0.979	0.894	0.937	0.915	1.000	0.974
B	0.008	0.011	0.021	0.106	0.062	0.085	0.000	0.026
mMDH-3								
(N)	60	87	48	99	98	100	100	42
A	0.992	0.994	0.969	0.828	1.000	1.000	1.000	0.869
B	0.008	0.006	0.031	0.172	0.000	0.000	0.000	0.131
mMEP-1								
(N)	53	62	48	100	100	100	100	37
A	1.000	0.992	0.979	1.000	0.960	1.000	1.000	1.000
B	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.008	0.010	0.000	0.040	0.000	0.000	0.000
MPI								
(N)	60	87	48	100	100	100	100	53
A	0.900	0.897	0.896	1.000	1.000	1.000	1.000	1.000
B	0.100	0.103	0.104	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEP-LT								
(N)	60	87	48	100	100	96	96	53
A	0.992	1.000	1.000	1.000	1.000	1.000	0.984	1.000
B	0.008	0.000	0.000	0.000	0.000	0.000	0.016	0.000
PEPA								
(N)	60	87	48	100	100	100	100	37
A	0.833	0.874	0.885	0.950	1.000	1.000	0.970	1.000
B	0.167	0.126	0.115	0.050	0.000	0.000	0.030	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PEPD								
(N)	60	87	48	100	100	100	100	53
A	0.975	0.989	0.990	1.000	1.000	1.000	1.000	1.000
B	0.025	0.011	0.010	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGDH								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGK-2								
(N)	60	87	47	100	100	100	100	53
A	0.575	0.592	0.596	0.415	0.315	0.200	0.350	0.472
B	0.400	0.356	0.394	0.555	0.680	0.800	0.650	0.519
C	0.008	0.034	0.011	0.005	0.000	0.000	0.000	0.000
D	0.017	0.017	0.000	0.025	0.005	0.000	0.000	0.009

Table 9b (cont.)

	Collection							
Locus	10	11	12	13	14	15	16	17
PGM-2								
(N)	60	87	48	100	**100**	100	**100**	53
A	0.983	0.994	0.969	1.000	0.855	1.000	0.615	1.000
B	0.000	0.006	0.021	0.000	0.145	0.000	0.385	0.000
C	0.017	0.000	0.010	0.000	0.000	0.000	0.000	0.000
PNP								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**1.000**
sSOD-1								
(N)	60	87	48	100	100	100	100	51
A	0.842	0.914	0.875	0.640	0.860	0.930	0.850	0.696
B	0.117	0.046	0.052	0.360	0.140	0.070	0.150	0.304
C	0.042	0.040	0.073	0.000	0.000	0.000	0.000	0.000
sSOD-2								
(N)	41	9	35	100	100	82	100	1
A	1.000	**1.000**	1.000	1.000	1.000	1.000	1.000	1.000
TPI-1								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-2								
(N)	60	87	48	100	100	100	100	53
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI-4								
(N)	60	87	48	100	100	100	100	37
A	0.908	0.983	0.937	1.000	1.000	1.000	1.000	1.000
B	0.092	0.017	0.062	0.000	0.000	0.000	0.000	0.000

Table 10. Average heterozygosity and percentage of loci polymorphic based on 59 loci.

Collection	Mean Sample Size per Locus	Mean Alleles per Locus	Percentage of Loci Polymorphic *	Mean Heterozygosity Direct-Count	Hdywbg Exp. **
1. Dry Cr. 89	81.7 .	1.5	3.9	0.055	0.057
	(1.4)	(0.1)		(0.017)	(0.018)
2. Logy Cr. 90	74.0	1.4	30.5	0.056	0.057
	(1.8)	(0.1)		(0.018)	(0.018)
3. Satus Cr. 90	94.1	1.4	32.2	0.062	0.060
	(2.7)	(0.1)		(0.019)	(0.018)
4. Yakima Hat. 90	46.3	1.5	39.0	0.069	0.067
	(0.9)	(0.1)		(0.020)	(0.018)
5. Wapatox 89	154.9	1.9	50.8	0.059	0.058
	(1.8)	(0.2)		(0.018)	(0.018)
6. Wapatox 90	91.4	1.5	40.7	0.052	0.053
	(2.5)	(0.1)		(0.018)	(0.018)
7. Nile Pond 90	43.3	1.4	27.1	0.054	0.054
	(1.7)	(0.1)		(0.018)	(0.017)
8. Roza 89	52.9	1.6	44.1	0.061	0.065
	(0.7)	(0.1)		(0.018)	(0.018)
9. Prosser 89-l	74.5	1.7	44.1	0.061	0.060
	(1.0)	(0.1)		(0.018)	(0.017)
10. Prosser 89-2	56.9	1.6	45.8	0.060	0.072
	(1.2)	(0.1)		(0.016)	(0.019)
11. Prosser 89-3	83.3	1.7	47.5	0.056	0.058
	(1.9)	(0.1)		(0.017)	(0.018)
12. Prosser 89-4	46.4	1.7	50.8	0.060	0.069
	(0.8)	(0.1)		(0.016)	(0.017)
13. Goldendale 90	99.2	1.4	27.1	0.072	0.076
	(0.6)	(0.1)		(0.020)	(0.021)
14. Spokane 90	99.8	1.4	30.5	0.068	0.068
	(0.1)	(0.1)		(0.019)	(0.019)
15. Tokul 90	99.3	1.2	18.6	0.058	0.060
	(0.4)	(0.1)		(0.019)	(0.020)
16. S. Tacoma 90	98.7	1.3	25.4	0.067	0.068
	(0.8)	(0.1)		(0.019)	(0.019)
17. Naches Hat. 90	48.7	1.3	22.0	0.060	0.060
	(1.2)	(0.1)		(0.020)	(0.019)

* A locus is considered polymorphic if more than one allele was detected

** Unbiased estimate (Nei 1978)

Genetic differences within collections

The nonduplicated loci were tested for agreement to Hardy-Weinberg equilibrium expectations (duplicated isoloci cannot be tested because we cannot tell how the genetic variation is partitioned among the two loci) (Table 11). Out of the 83 total tests in the non Prosser wild steelhead, all but five loci were in agreement. This is similar to what is expected due to chance with significance at the p = 0.05 level. In contrast, eleven loci in three of the four Prosser collections (numbers 2-4) had a deficit of heterozygotes--an indication of multiple stocks contributingtothe collections. No differences were found in the first Prosser collection (89-l).

Table 11. Significant deviations of genotype counts from Hardy-Weinberg equilibrium expectations within Yakima steelhead collections and four rainbow trout strains.

Collection	Loci	Reason
Wild steelhead		
Dry Creek 90	none	
Satus Creek 90	none	
Logy Creek 90	none	
Wapatox 89	*ADA-1	deficit of heterozygotes
	ADA-2*, MPI*	rare genotype
Wapatox 90	PGM-2*	rare genotype
Roza 89	mIDHP-2*	deficit of heterozygotes
Prosser 89-l	none	
Prosser 89-2	ADA-1 *, CK-A1*,	
	mIDHP-2*, LDH-B2*	deficit of heterozygotes
	ADA-2*	rare genotype
Prosser 89-3	ADA-1 *, PGK-2*	deficit of heterozygotes
	IDDH-2*,	rare genotype
Prosser 89-4	LDH-B2*, PEPA*	deficit of heterozygotes
	CK-A1*	rare genotype
Hatchery Steelhead		
Yak. Hat. 90	sIDHP-2*	excess of heterozygotes
Nile Pond 90	*SOD-1	deficit of heterozygotes
Hatchery Rainbow		
Goldendale 90	ADA-1 *	possible nongenetic variation
Spokane 90	ADA-1 •	possible nongenetic variation
South Tacoma 90	*ADA-1	possible nongenetic variation
Tokul Cr. 90	ADA-1 •	possible nongenetic variation
	mAH-3*	rare genotype
Naches	IDDH-1*	deficit of heterozygotes

Genotype frequencies at the locus ADA-l* did not conform to the expected Hardy-Weinberg proportions in all four hatchery rainbow trout strains, although this locus in the Goldendale at Naches collection was in agreement. But, no *104, *105 and *113 alleles were observed in the Naches collection, whereas these alleles were common in the other rainbow trout collections. Therefore, the *104, *105, and *113 alleles may not represent real genetic variation (Also see Kobayashi et al. 1984). Genotypes with these alleles were zeroed for the genetic distance comparisons.

We also tested the four Prosser collections for departures from gametic equilibrium. Gametic disequilibrium is nonrandom associations of alleles and is an indication of multiple reproductive groups in a collection. Significant gametic disequilibrium was observed in two of the four collections (Prosser89-1, Lambda=74.6, 45df, p<0.01; Prosser89-2, Lambda=107.8, 45df, p<0.001; Prosser89-3, Lambda=48.3, 45df, 'not significant; Prosser89-4, Lambda=57.5, 45df, not significant).

Genetic differences among collections

Two genetic distance measures (Nei's unbiased and Cavalli-Sforza and Edwards' chord distance) based on 59 loci were calculated (Table 12) and the latter measure was used to build a dendrogram (unweighted pair group method) to visualize genetic relationships (Fig.5).

Table 12. Genetic distance measures. Below diagonal: Nei (1978) unbiased genetic distance. Above diagonal: Cavalli-Sforza & Edwards (1967) chord distance.

	Collection	1	2	3	4	5	6	7	8
1	Dry Cr. 89	*****	0.046	0.044	0.044	0.060	0.062	0.072	0.063
2	Logy Cr. 90	0.000	*****	0.043	0.052	0.059	0.058	0.064	0.072
3	Satus Cr. 90	0.000	0.001	*****	0.046	0.060	0.063	0.068	0.068
4	Yakima Hat. 90	0.000	0.000	0.000	*****	0.059	0.060	0.069	0.063
5	Wapatox 89	0.001	0.001	0.001	0.001	*****	0.045	0.058	0.056
6	Wapatox 90	0.001	0.000	0.001	0.001	0.000	*****	0.047	0.066
7	Nile Pond 90	0.002	0.001	0.002	0.001	0.001	0.000	*****	0.073
8	Roza 89	0.001	0.002	0.001	0.002	0.001	0.002	0.002	*****
9	Prosser 89-1	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.000
10	Prosser 89-2	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.000
11	Prosser 89-3	0.000	0.001	0.001	0.001	0.000	0.001	0.001	0.000
12	*Prosser 89-4*	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.000
13	Goldendale 90	0.034	0.033	0.031	0.031	0.028	0.030	0.027	0.023
14	Spokane 90	0.017	0.018	0.016	0.015	0.014	0.015	0.012	0.013
15	Tokul 90	0.032	0.032	0.030	0.030	0.027	0.029	0.025	0.022
16	S Tacoma 90	0.027	0.028	0.026	0.025	0.023	0.024	0.019	0.022
17	Naches Hat. 90	0.030	0.030	0.028	0.028	0.025	0.027	0.024	0.020

	collection	9	10	11	12	13	14	15	16	17
1	Dry Cr. 89	0.050	0.068	0.054	0.070	0.181	0.156	0.174	0.164	0.174
2	Logy Cr. 90	0.060	0.075	0.060	0.075	0.186	0.161	0.177	0.168	0.178
3	Satus Cr. 90	0.054	0.073	0.059	0.073	0.180	0.155	0.174	0.162	0.173
4	Yakima Hat. 90	0.050	0.059	0.056	0.066	0.174	0.151	0.167	0.158	0.169
5	Wapatox 89	0.045	0.062	0.047	0.052	0.157	0.134	0.153	0.143	0.151
6	Wapatox 90	0.053	0.069	0.054	0.063	0.167	0.144	0.160	0.149	0.157
7	Nile Pond 90	0.064	0.076	0.064	0.071	0.164	0.133	0.155	0.135	0.153
8	Roza 89	0.049	0.051	0.049	0.050	0.151	0.136	0.143	0.140	0.144
9	Prosser 89-l	*****	0.056	0.039	0.054	0.159	0.138	0.152	0.141	0.151
10	Prosser 89-2	0.000	*****	0.052	0.050	0.142	0.134	0.138	0.142	0.137
11	Prosser 89-3	0.000	0.000	*****	0.049	0.155	0.136	0.151	0.139	0.148
12	Prosser 89-4	0.000	0.000	0.000	*****	0.145	0.126	0.139	0.133	0.138
13	Goldendale 90	0.027	0.022	0.027	0.022	*****	0.109	0.092	0.129	0.045
14	Spokane 90	0.014	0.012	0.014	0.011	0.011	*****	0.106	0.096	0.103
15	Tokul 90	0.026	0.022	0.026	0.021	0.008	0.012	*****	0.124	0.084
16	S Tacoma 90	0.023	0.021	0.022	0.019	0.014	0.006	0.015	*****	0.122
17	Naches Hat. 90	0.023	0.020	0.024	0.019	0.000	0.010	0.007	0.013	*****

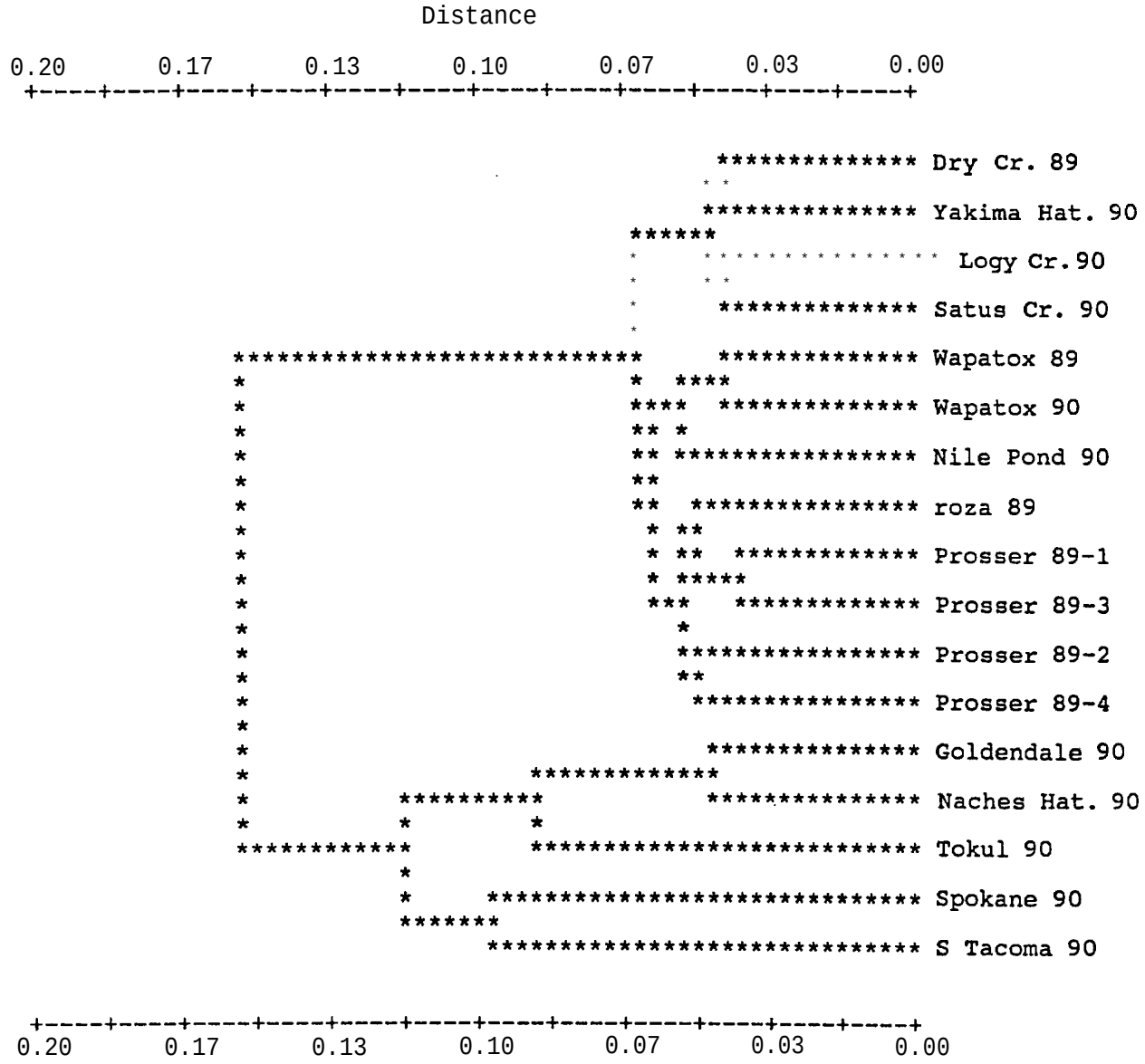

Fig.5. Dendrogram of genetic distances (Cavalli-Sforza & Edwards (1967) chord distance).

Heterogeneity chi-square tests were performed to identify reproductive isolation among collections at some locations (Tables 13a-d). Significant heterogeneity was observed ($p<0.00001$) among Satus Cr., Dry Cr., and Logy Cr. collections tested as a group. The Wapatox 1989 and 1990 collections were also different ($p<0.022$). Yakima Hatchery adults were different from Nile Pond smolts ($p<0.003$). significant changes in allele frequencies were also evident in the temporal collections at Prosser ($p<0.028$).

Table 13a. Heterogeneity chi-square: **Satus** Cr. X Dry Cr. X Logy Cr.

LOCUS[1]	NO. OF ALLELES	CHI-SQUARE	D.F.	P
sAAT-1,2	2	2.317	2	0.31396
sAAT-3	2	3.680	2	0.15883
mAAT-1	2	6.464	2	0.03948
ADA-l	2	0.641	2	0.72579
ADA-2	2	6.442	2	0.03992
ADH	2	2.389	2	0.30280
SAH	3	9.801	4	0.04391
ALAT	2	1.325	2	0.51548
CK-A2	2	4.792	2	0.09107
IDDH-2	2	2.211	2	0.33108
mIDHP-2	2	1.211	2	0.54567
sIDHP-1	3	4.794	4	0.30912
sIDHP-2	8	26.688	14	0.02113
LDH-B2	3	6.169	4	0.18685
sMDH-A1,2	2	0.824	2	0.66240
sMDH-B1,2	4	7.422	6	0.28361
mMEP-1	2	8.637	2	0.01332
MPI	3	5.085	4	0.27867
PEPA	2	8.677	2	0.01306
PEPD	2	2.585	2	0.27459
PGK-2	3	1.612	4	0.80665
sSOD-1	3	18.024	4	0.00122
TPI-4	2	7.852	2	0.01972
(TOTALS)		139.643	74	0.00001

[1] **sIDHP-1,2** was treated as two independent loci for this analysis,

Table 13b. Heterogeneity-chi-square: Wapatox 89 X Wapatox 90

LOCUS[1]	NO. OF ALLELES	CHI-SQUARE	D.F.	P
sAAT-1,2	3	3.842	2	0.14645
sAAT-3	2	1.593	**1**	0.20685
mAAT-1	2	2.531	**1**	0.11166
ADA-l	2	0.552	**1**	0.45742
ADA-2	3	1.928	2	0.38128
ADH	2	0.630	1	0.42721
sAH	2	0.073	1	0.78642
mAH-1	2	3.941	1	0.04712
mAH-3	2	0.485	1	0.48600
ALAT	4	1.486	3	0.68549
CK-Cl	2	1.148	**1**	0.28394
GPI-B2	2	1.593	1	0.20685
GPI-A	3	1.310	2	0.51950
G3PDH-1	2	**0.110**	1	0.74019
IDDH-2	2	3.169	1	0.07504
mIDHP-1	2	**0.033**	1	0.85677
mIDHP-2	3	6.005	2	0.04968
sIDHP-1	3	1.370	2	0.50411
sIDHP-2	8	11.626	7	0.11355
LDH-B2	2	0.007	1	0.93119
LDH-C	2	0.110	1	0.74019
sMDH-A1,2	2	0.221	1	0.63850
sMDH-B1,2	5	2.234	4	0.69286
mMDH-1	2	2.510	1	0.11312
mMDH-2	2	1.022	1	0.31211
mMEP-1	2	0.628	1	0.42823
MPI	3	2.670	2	0.26318
PEPA	2	5.023	**1**	0.02502
PEPD	2	10.522	**1**	0.00118
PGK-2	4	4.067	3	0.25429
PGM-2	3	1.268	2	0.53038
sSOD-1	3	1.315	2	0.51820
TPI-4	2	1.755	1	0.18521
(TOTALS)		76.778	**54**	**0.02249**

[1] **sIDHP-1,2** was treated as two independent loci for this analysis.

Table 13c. Heterogeneity chi-square: Yakima Hat. Adults X Nile Pond smolts.

LOCUS[1]	NO. OF ALLELES	CHI-SQUARE	D.F.	P
sAAT-1,2	3	3.026	2	0.22022
sAAT-3	2	5.975	1	0.01451
mAAT-1	2	0.185	1	0.66734
ADA-l	2	8.499	1	0.00355
ADA-2	2	0.622	1	0.43032
sAH	2	0.308	1	0.57873
mAH-1	2	0.247	1	0.61925
mAH-3	2	0.309	1	0.57858
ALAT	3	12.468	2	0.00196
CK-Al	2	1.047	1	0.30619
CK-Cl	2	0.644	1	0.42228
G3PDH-1	2	0,985	1	0.32097
IDDH-2	2	1.577	1	0.20920
mIDHP-2	2	1.062	1	0.30267
sIDHP-1	2	1.026	1	0.31119
sIDHP-2	5	4.036	4	0.40111
LDH-B2	3	1.670	2	0.43393
LDH-C	2	2.105	1	0.14683
sMDH-A1,2	2	1.026	1	0.31119
sMDH-B1,2	4	1.539	3	0.67337
MPI	2	0.721	1	0.39593
PEPA	2	2.059	1	0.15129
PGK-2	4	7.693	3	0.05281
PGM-2	2	5.027	1	0.02496
sSOD-1	3	0.029	2	0.98550
TPI-4	2	0.967	1	0.32549
(TOTALS)		64.851	37	0.00311

[1] sIDHP-1,2 was treated as two independent loci for this analysis.

Table 13d. Heterogeneity chi-square: Prosser 1989 temporal collections.

LOCUS[1]	NO. OF ALLELES	CHI-SQUARE	D.F.	P
sAAT-1,2	3	3.394	6	0.75807
sAAT-3	2	0.852	3	0.83688
mAAT-1	2	1.628	3	0.65306
ADA-l	2	6.911	3	0.07479
ADA-2	3	5.268	6	0.50994
ADH	2	4.655	3	0.19890
SAH	3	2.864	6	0.82566
mAH-1	2	9.368	3	0.02478
ALAT	4	7.999	9	0.53422
CK-Al	2	15.461	3	0.00146
CK-Cl	2	2.730	3	0.43523
GPI-Bl	2	4.269	3	0.23386
GPI-B2	2	2.130	3	0.54577
GPI-A	3	7.298	6	0.29414
G3PDH-1	2	6.101	3	0.10682
IDDH-1	2	1.061	3	0.78661
IDDH-2	2	1.263	3	0.73795
mIDHP-1	2	3.112	3	0.37473
mIDHP-2	2	6.863	3	0.07641
sIDHP-1	3	7.640	6	0.26570
sIDHP-2	8	20.037	21	0.51889
LDH-Al	2	2.537	3	0.46858
LDH-B2	2	3.799	3	0.28403
LDH-C	2	1.450	3	0.69384
sMDH-A1,2	3	7.447	6	0.28148
sMDH-B1	2	3.540	3	0.31560
sMDH-B2	8	30.115	21	0.08970
mMDH-1	2	2.969	3	0.39645
mMDH-2	2	6.751	3	0.08026
mMEP-1	3	5.376	6	0.49663
MPI	2	0.851	3	0.83722
PEP-LT	2	3.540	3	0.31560
PEPA	2	2.021	3	0.56813
PEPD	3	4.479	6	0.61213
PGK-2	4	6.248	9	0.71481
PGM-2	3	6.207	6	0.40046
sSOD-1	3	14.136	6	0.02815
TPI-4	2	8.759	3	0.03267
(TOTALS)		231.128	192	0.02815

[1] **sIDHP-1,2** and **sMDH-B1,2** were treated as two independent loci for this analysis.

DISCUSSION

Genetic diversity among Yakima steelhead

We identified significant genetic diversity among steelhead collections within the Yakima River. Adults collected at the Yakima Hatchery most closely resembled the three Satus Creek collections, whereas hatchery smolts from Nile Pond were most similar to the two Wapatox collections. Three indications of significant heterogeneity within the temporal Prosser collections (a deficit of heterozygotes and gametic disequilibrium within collections, and the heterogeneity chi-square test) were observed. Multiple stocks appear to be outmigrating past Prosser simultaneously.

Gene similarity between Yakima steelhead and hatchery rainbow

The four Washington Department of Wildlife rainbow trout strains are very different from Yakima steelhead. However, these differences are mainly due to frequencies of alleles, and with the exception of IDDH-l*, alleles present in the hatchery rainbow were also observed in the steelhead smolt collections.

Comparison of Satus Creek with Skamania Hatchery

I used allelic data for Skamania Hatchery from Milner and Teel (1979) and Schreck et al. (1986) and compared it to Satus Creek. Fifteen locus systems (duplicate isolocus systems counted as one) were comparable. Six loci were very different and several loci had common alleles that were absent in the other population. GPI-A* was monomorphic in Satus Creek but, the 89* allele was at a frequency of 0.08 to 0.02 in Skamania. G3PDH-1* was monomorphic in Satus Creek, but the *80 allele had a frequency of 0.14 to 0.05 in Skamania. The LDH-B2*76 frequency of 0.36 in Satus Creek is typical of the inland race of steelhead, whereas, Skamania had a frequency of 0.12. I found variation at MPI* in Satus Creek, the *95 allele frequency was (0.09), whereas Skamania has no variation at this locus. However, Schreck et al. (1986) did not observe any variation at this locus in the Yakima samples they assayed. Milner and Teel (1979) found PEPA* variation in Skamania at low frequency (0.02 for the *111 allele) whereas Schreck et al. (1986) observed no variation at this locus. I found the frequency of the *111 allele in Satus Creek to be over 16%. sSOD-1* is another locus that distinguishes the inland and coastal races of rainbow trout. Satus Creek had a *152 and *38 allele frequency of 2% and 10%, respectively, whereas Skamania had a frequency of the *152 allele of 30% and did not have the *38 allele.

CONCLUSIONS

Restricted gene flow exists among steelhead populations within the Yakima River. This genetic heterogeneity does not appear to be caused by the past stocking of Skamania strain steelhead in some areas of the basin. The lack of alleles typical of the Skamania strain in the Yakima collections indicates that these hatchery fish likely did not contribute successfully to the present steelhead populations. Satus Creek steelhead, as well as steelhead from other areas, appear to contain native gene pools.

SUMMARY RECOMMENDATIONS

Standardization of genetic data from Yakima and Klickitat steelhead to other Columbia River and Snake River areas needs to continue so that the magnitude of within river genetic diversity' can be compared to among basin diversity. We developed an initial genetic screening protocol to identify allelic variation. Initial standardization work with NMFS geneticist should be expanded to include additional steelhead researchers.

This initial analysis of Yakima River steelhead identified significant genetic heterogeneity among locations. Analysis of 1991 collections (see 1991 steelhead substock identification plan) will help determine if this heterogeneity is a function of low effective population sizes and represent unstable substock characteristics or if it represents historical restrictions to gene flow.

Collections of smolts and adults from the Klickitat River in the winter and spring of 1991 will allow an initial look (by WDF) at summer steelhead from this river. Data from four suspected winter run steelhead will be combined with 1991 collections to begin the genetic profile of this run-time component.

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Appendix 3. WDF baseline screening protocol for rainbow trout.

Muscle

CAME6.8 [THICK GEL 35mm origin, 5 1/2 hrs @ 250V (max 90 mA)]
FH
IDHP & PGDH
ADA
PGK scrape and stain AK
ALAT
PEP-LT & PEPD
MEP & PGM a+c20mg oxaloacetate
AAT c only
G3PDH a+c (c only if no anodal slices)

RW (nonzap) [THICK GEL 35mm origin, 5 hrs @ 80mA (max 400V)] LKB
ESTD scrape & stain LDH
AAT
GPI
G3PDH
CK
PEPB

Tris-Gly [THICK GEL 35mm Origin, 5 1/2 hrs @ 600V (max 90 mA)]
LKB
HAGH
ADA
ALAT
GPI
TPI
MPI & CK
PGM

CAME6.8N [Add 8ml of NAD buffer solution (15mg/ml) to the gel immediately before degassing -- and add 2ml to cathodal electrode tray]
[THICK GEL 35mm origin, 5 1/2 hrs @ 250V (max 90 mA)]
AAT a+c (if extra anodal slice is available)
AH
GAPDH
MEP (40 mg oxaloacetate)
G3PDH
SOD
MDH
IDHP

Appendix 3. (cont.) Rainbow trout baseline screening protocol cont.

Heart
EBT (nonzap) [THICK GEL 35mm origin, 5 hrs @ 80mA (max 400 V)]
AAT
PEPB & PEPA
PEPD
SOD
TPI
GR
MPI

Liver
CAME6.8 [THICK GEL 35mm origin, 5 1/2 hrs @ 250V (max 90 mA)]
MDH
AH
PGK
IDHP
GDA
ADH (cathode only)
MEP
PGM (a+c)

RW (nonzap) [THIN GEL 35mm origin, 5 hrs @ 80mA (max 400V)] LKB
EST
IDDH
bGLUA
AAT
ADH (cathode only)
LDH

TC-4 [THIN GEL 35mm origin, 5 1/4 hrs @ 250V (max 90 mA)]
aMAN
bGLUA a+c
bGALA 9.5 USE TRIS
GDA
PEPB (cathode only)
MEP (if slice is available)

Eye
Tris-Gly [THICK GEL 35mm origin, 5 1/2 hrs @ 600V (max.90 mA)]
LKB
FBALD
AAT
PNP
CK
TPI
MPI
LDH

Appendix 3. (cont.) Rainbow trout baseline screening protocol cont.

cAM6.8 [THICK GEL 35mm origin, 5 1/2 hrs @ 250V (max 90 mA)]
LDH
GAPDH
IDHP & PGDH
MPI
GR
PNP
CK

Appendix 4. Gel and electrode buffers used for rainbow trout electrophoresis.

Buffer

CAME6.8 (modified from Clayton and Tretiak 1972)
Electrode
0.04M citric acid
0.005M EDTA
Adjust pH with N-(3-aminopropyl) morpholine
Gel 1 in 20 dilution of electrode and readjust pH

CAM6.8 modified CAME6.8 by not adding the EDTA

CAME6.8N modified CAME6.8 by adding 0.15 mg NAD per ml of gel buffer and 30mg NAD to the cathodal electrode tray.

RW (from Ridgway et al. 1970)
Electrode
0.06M lithium hydroxide
0.3M boric acid
Gel
0.03M tris
0.005M citric acid
Add electrode buffer (1% of final gel volume)

EBT (from Boyer et al. 1963)
Electrode
0.18M tris
0.1M boric acid
0.004M EDTA
Gel 1 in 4 dilution of electrode

Tris-Gly (modified from Holmes and Masters 1970)
Electrode and Gel are the same solution
3.0g/l tris
14.4g/l glycene

TC-4 (buffer "a" of Schaal and Anderson 1974)
Electrode (final pH 5.8)
27g/l tris
18.1g/l citric acid (monohydrate)
Gel 1:27.5 dilution of electrode

Appendix 5. Allele mobilities of rainbow trout and steelhead genetic variation observed at each locus on different tissue and buffer combinations (WDF 14 October 1990). [] = NMFS allele not identified in populations WDF has studied. NS= not scorable on this tissue/buffer combination. () = suspect variation not used in analysis. %% = no allele for this number/letter code.

Steelhead Relative Allele Mobilities

LOCUS	T	Buffer	A 1	B 2	C 3	D 4	E 5	F 6	G 7	H 8	I 9
sAAT1,2	M	Tris-Gly	100	112	90						
	M	RW	100	109	92		101				
	M	CAM6.1	100	125	NS	113					
	HM	CAME6.8	100	125	95		103				
	H	EBT	100	114	90						
sAAT-3	E	Tris-Gly	100	69	[109]						
	L	CAME6.8	100								
	L	RW	100								
sAAT-4	L .	CAME6.8	100								
	L	RW	100	105	110						
mAAT-1	MH	CAME6.8	-100	-110							
	M	CAM6.1	-100	-110							
	M	Tris-Gly	-100	-110							
mAAT-2	MH	CAME6.8	-100	(-90)							
	M	CAM6.1	-100	(-90)							
mAAT-3	M	CAM6.1	-100								
	MH	CAME6.8	-100								
ACR	H	EBT	100								
	M	Tris-Gly	100								
ADA-l	M	CAME6.8	100	85	81	104	105	113			
	E	CAM6.8	100	85	81	104	105	113			
	M	Tris-Gly	100	93	(#2)	102	105	113			
ADA-2	M	Tris-Gly	100	106	90	110					
	M	CAME6.8	100	NS		105					
ADH	L	cAM6.1	-100	-78							
	L	CAME6.8	-100	-78	-50						
	L	RW	-100	-82		-5					
	L	Tris-Gly	-100	-21							
sAH	L	CAME6.8	100	85	%%	72					
	L	CAM6.1	100	85							
mAH-1	H	CAME6.8	100	55							
	M	CAME6.8	100								
mAH-2	H	CAME6.8	100	(186)							
	ME	CAME6.8	100								
mAH-3	H	CAME6.8	100	(122)	(114)						
	M	cAMEi6.8	100								
mAH-4	H	CAME6.8	(100)								
	M	CAME6.8	(100)								
AK	M	CAME6.8	100								
ALAT	M	CAME6.8	100	105		88					
	M	Tris-Gly	100	106	111	91					
CK-Al	M	RW	100	67							
	M	CAME6.8	100	50							
	M	Tris-Gly	100	67	75						
CK-A2	M	RW	100	(108)							
	M	CAME6.8	100								
	M	Trie-Gly	100								
CK-B	E	Tris-Gly	100	[97]							
CK-Cl	E	Tris-Gly	100	105	(98)						
CK-C2	E	Tris-Gly	100								

Appendix 5. (cont.)

			Steelhead Relative Allele Mobilities												
			A	B	C	D	E	F	G	H	I	J	K	L	
LOCUS	T	Buffer	1	2	3	4	5	6	7	8	9	10	11	12	
DIA1	E	CAM6.8	100	109	(128)										
ESTD	LM	RW	100	102											
	M	Tris-Gly	100												
EST-2	L	RW	100	110	(105)										
FBALD-3	E	Tris-Gly	100												
FBALD-4	E	Tris-Gly	100												
FH	M	CAME6.8	100	84											
bGALA	L	CAM6.1	100												
	ML	Tris-Gly	100												
	L	TC-4	100	80											
	L	RW	100												
	L	CAME6.8	100												
GAPDH-1	M	CAME6.8N	100												
GAPDH-2	H	CAME6.8N	100												
GAPDH-3	HM	CAME6.8N	100	33	120										
GAPDH-4	E	CAME6.8	100												
GAPDH-5	E	CAME6.8	100												
GDA-1	L	Tris-Gly	(100)	NS	NS	(110)									
	L	TC-4	(100)	(120)	(80)		(1 8 3)								
	L	CAMFa6.8	(100)	(120)	(80)		(183)								
GDA-2	L	Tris-Gly	(100)												
	L	TC-4	(100)	(115)	(90)	(55)									
	L	CAME6.8	(100)	(115)	(90)	(55)									
bGLUA	L	CAM6.1	100	-39	-11	93									
	L	TC-4	100	-39	-11	93									
	L	RW	100	77	85	93									
	L	CAME6.8	100	2	10	93									
GPI-B1	M	RW	100	142	[130]	15	[25]								
	M	Tris-Gly	100	148		15									
GPI-B2	M	RW	100	60	150										
	M	Tris-Gly	100	60	150										
GPI-A	M	Tris-Gly	100	115	89	[107]									
	M	RW	100	105	93										
GR	H	CAME6.8	100	(115)											
	E	CAM6.8	100												
	MH	EBT	100												
G3PDH-1	M	CAME6.8	-100	80											
	M	CAM6.1	-100	-7											
G3PDH-2	M	CAME6.8	-100	150											
	M	CAM6.1	-100												
G3PDH-3	H	CAME6.8N	100	64											
G3PDH-4	H	CAME6.8N	100	124											
	M	CAME6.8	100	124											
HAGH	M	Tris-Gly	100	70											
IDDH-1	L	RW	100	200	15	400									
IDDH-2	L	RW	100	143	5										
mIDHP-1	MH	CAME6.8	100												
mIDHP-2	MH	CAME6.8	100	144	162	67									
sIDHP-1	MH	CAME6.8	100		122	71				116					
sIDHP-1,2	L	cAM6.1	100	4	129	72	[?]	118		121					
	L	CAME6.8	100	42	121			123	40	116	58	74	27	80	
	E	CAM6.8	100	42	121	72		123	40	116	58	74	27	80	
LDH-A1	M	RW	100	420											
	M	Tris-Gly	100												
	M	cAM6.1	-100												

Appendix 5. (cont.)

Steelhead Relative Allele Mobilities

Locus	T	Buffer	A 1	B 2	C 3	D 4	E 5	F 6	G 7	H 8	I 9
LDH-A2	M	RW	100								
	M	Tris-Gly	100								
	M	cAM6.1	-100								
LDH-B1	E	CAM6.8	100								
	M	RW	100								
	E	Tris-Gly	100								
LDH-B2	E	Tris-Gly	100	76	113	[97]					
	LM	RW	100	76	113						
LDH-C	E	Tris-Gly	100	9	5						
	E	CAM6.8	100	97							
aMAN	L	Tris-Gly	100	115	85						
	L	TC-4	100								
sMDH-A1,2	H	CAME6.8	100	155	37	120	49				
	LM	CAM6.1	100	210	-15						
	M	CAME6.8	100	155	37						
sMDH-B1,2	H	CAME6.8	100	78	116	83	92	120	104	125	
	M	CAM6.1	100	75	115	81		119			
	M	CAME6.8	100	77	113	84	95	118	104	125	
	M	Tris-Gly	100	64	130	(#2)		(#3)			
mMDH-1	HM	CAME6.8	-100								
mMDH-2	HM	CAME6.8	100								
	LM	cAM6.1	100								
mMDH-3	HM	CAME6.8	100	185	50						
	M	CAME6.8	100								
	LM	CAM6.1	100								
ME	H	CAME6.8N	100	110							
mMEP-1	M	CAME6.8	100	90	36	115					
mMEP-1,2	H	CAME6.8	100	90	36	115					
sMEP-1	MH	CAME6.8	100	83	98	102					
	L	CAME6.8	100	83			115				
	L	TC-4	100	83							
sMEP-2	L	TC-4	(100)								
	LMH	CAME6.8	(100)								
MPI	H	EBT	100	95	104	90					
	EL	Tris-Gly	100	95	104						
	E	CAM6.8	100	96	104						
NTP	M	RW	100	135	161	76					
PEPA	M	CAME6.8	100	122	79						
	H	EBT	100	111	93	%%	119				
	M	CAM6.1	100	138	75						
	MLE	Tris-Gly	100	111	92						
PEPB-1	MH	EBT	100	134							
	L	TC-4	-100	[#5]	(110)	%%	-50				
	M	Tris-Gly	100	131							
	M	RW	100	118	(74)						
PEPD-1	M	CAME6.8	100	94	110						
	H	EBT	100	93	105						
	M	Tris-Gly	100	93	111						
PGDH	M	CAME6.8	100								
	E	CAM6.8	100								
PGK-1	M	CAME6.8	-100								
PGK-2	H	CAM6.8	100	115	144	136					
	ME	CAME6.8	100	115	144	136					
PGM-1	ME	CAME6.8	-100	null	-85	-140					
	M	Tris-Gly	100	null							
PGM-lr	L	CAME6.8	null	100							

Appendix 5. (cont.)

Steelhead Relative Allele Mobilities

Locus	T	Buffer	A 1	B 2	C 3	D 4	E 5	F 6	G 7	H 8	I -	L
PGM-2	M	CAM6.8	-100	-120	5	[-167]						
	ME	CAME6.8	-100	-120	5	[-167]						
	M	EBT	100	84								
	M	RW	100	85	120							
	M	Tris-Gly	100	81	120							
PNP	E	CAM6.8	100	107								
	E	Ttis-Gly	100	102								
PNPl-1	E	CAM6.8	100	82								
PNPl-2	E	CAM6.8	100									
sMEP-1	M H	CAME6.8	100	83	98	102						
	L	CAME6.8	100	83			115					
	L	TC-4	100	83								
sMEP-2	L	TC-4	(100)									
	LMH	CAME6.8	(100)									
sSOD-1	L	CAM6.1	100	226	16							
	L	RW	100	152	38							
	L	Tris-Gly	100	154	42							
	H	EBT	100	152	38							
	H	CAME6.8	100	226	16							
sSOD-2	H	EBT	100									
	H	CAME6.8	100									
mSOD	H	EBT	100	148								
	H	CAME6.8	100	124								
TPI-1	MH	EBT	-100									
	ME	Tris-Gly	-100									
TPI-2	MH	EBT	-100	500								
	ME	Tris-Gly	-100	500								
TPI-3	MH	EBT	100	94	102	97						
	ME	Tris-Gly	100	96	102	98						
TPI-4	MH	EBT	100	101								
	ME	Tris-Gly	100	101								

YKFP GENETIC RISK ASSESSMENT

INTRODUCTION

The Northwest Power Planning Council's support for genetic conservation efforts is clearly enunciated in its 1987 Columbia River Basin Fish and Wildlife Proaram (Section 204, paragraph b). Probably the most significant aspect of the Council's commitment to genetic conservation is a stipulation that a genetic risk assessment be done in planning for any production project under the Council's purview. Accordingly, in early 1990 I wrote a genetic risk assessment (GRA) for the YKPP. This document was included in Appendix A of the YKPP Preliminary Design Report. The 1990 YKPP GRA was, to the best of our knowledge, the first genetic risk assessment written on any Columbia basin production program, and as such it may have a long term influence on GRA development, both in the region and outside (it has been fairly well distributed nationwide). However, it is just the first step in the genetic risk analysis process for the project. I acknowledged the preliminary nature of the document, and cited three reasons the GRA would need revisions: 1) lack of clear guidelines for genetic risk assessment development from the Council, 2) lack of data on which to assess risk, and 3) changes in risk status caused by adaptive management of the project. One additional reason for revision was created by the Council's response to the GRA. These four issues and our activities related to them will be discussed below; because adaptive management includes response to criticism, including criticism from the Council, adaptive management and Council response will be discussed together.

DEVELOPMENT OF NPPC GRA GUIDELINES

At the time the YRPP GRA was being prepared, the Council's monitoring and evaluation group (MEG) had been grappling with genetic risk assessment for several years, but no clear guidelines existed. An "Expert System" assessment software package was under development by Larry Riggs, a Council genetics consultant, but was not yet close to operational status. I did use a number of ideas from Riggs and MEG documents, however. Chief among these was the categorization of risk. Riggs had identified three categories of risk: 1) extinction, 2) loss of within population variability, and 3) loss of population identity (between population variability). I added a fourth category, domestication selection, which Riggs had considered an aspect of category 2. I also subdivided category 2 into two subclasses: 2a, loss of genetic variability through genetic drift; and 2b, loss of variability through non-representative broodstock selection. I did not use the concepts of production opportunities and categories outlined in Riggs (1990), however because: 1) there seemed to be a considerable amount of confusion surrounding the application of opportunity categories;

and 2) the YKPP, because of its experimental nature and strong commitment to adaptive management, did not seem to fit the opportunity classification well; i.e., experimental needs, rather than overall production goals, dictate the project design.

The **GRA** was rightly criticized by Riggs, under Council contract, for incompleteness with respect to the specifics of the actual production program. However, the YKPP GRA was in general well received by a broad readership and is still, in the continuing absence of Council guidelines, recommended as an example to follow when preparing a project GRA.

In late 1991, Larry Riggs, Lars **Mobrand,** Phil Roger, Chip **McConnaha, Willa** Nehlsen, and I met for three days to develop Council **GRA** guidelines. The individual sections of the **GRA** guidelines were assembled in March 1991, and a draft of the full guidelines is now being prepared. There are still some unresolved difficulties with the guidelines, so it is unclear when they will be ready for use.

Although the NPPC guidelines are not yet finalized, the general approach now planned is probably that which will appear in the final document, so the approach described in the current draft is worth describing here. The NPPC approach, called genetic impact assessment rather than genetic risk assessment, is carried out at two levels. Level 1 is a preliminary approach to be carried out during the development of a draft master plan for a **subbasin** production program. An essential part of the Level 1 assessment is identification of critical uncertainties and data needs. Once these required data are gathered, the more detailed Level 2 assessment is done, which details impacts associated with specific project operations and identifies monitoring needs. The assessment involves a number of specified steps involving the assignment of each stock/production scheme combination to an opportunity category (Riggs **1990),** and rating the genetic risk on four impact "yardsticks" corresponding to the four categories of genetic risk. The guidelines include allowable impact levels for each opportunity category:

	Impact Categories			
Opportunity Category	1	2	3	4
A	3	3	1	1
B	3	3	2	1
C	3	3	2	2
D	3	3	3	3
E	3	3	3	3
F	3	3	3	3

A scale of 1 to 5 is used, 1 being low impact and 5 high impact. If a production scheme exceeds the allowable impact level, it must be justified. There are two problems we see at the moment with this system: 1) having a reasonable number of regional geneticists agree on the table above, and 2) having some reasonably objective way of deciding what level of impact a given action might have; e.g., how is a planner to know if the project he proposes will cause an impact level for type 4 risk of 3 or 4? This type of guidance was to come from Riggs' Expert System, which was to offer a simulation of what a group of geneticists would say when confronted with these decisions, but development of the Expert System has been suspended. Thus, these impact ratings have to be done "manually", without benefit, at least for the time being, of a consistent approach.

Despite these developmental problems, with the heavy participation of YKPP planners in the NPPC GRA planning process, it should be possible to generate a revised GRA acceptable to the Council whenever it is required. This document would have to be a mixture of Level 1 and Level 2, as some critical uncertainties have been addressed, but others remain (see below).

NEW INFORMATION

The 1990 GRA was necessarily vague on risk levels for production strategies because design of the program was to depend on the genetic diversity found to exist in the basin. Specifically, risks to substocks depended on how many substocks were present, and how well physical facilities and production plans fit in with substock structure. At the time the GRA was written, no results from WDF's genetic sampling of Yakima and Klickitat stocks were available. The situation is now much different for some substocks. Although there is a chance that future monitoring may change the situation, it appears there are only three Yakima spring chinook stocks: American, Naches, and Yakima. Since existing plans allowed for existence of all three, we can produce a Level 2 GRA on Yakima spring chinook. Progress has been slower on Yakima summer steelhead. Existing plans call for hatchery supplementation of Yakima and Naches stocks. We still don't know if multiple substocks exist in one or both of these areas. The discovery of a second Yakima fall chinook substock in Marion Drain complicates fall chinook operational planning. Some solid production options need to be developed before a Yakima fall chinook GRA can refined (see below). Substock ID work is still at too preliminary a level in the Klickitat to revise the GRA as it pertains to Klickitat substocks.

Another source of new information bearing on the genetic risk issue is two new papers dealing with extinction risk: one a synthesis paper in draft (Emlen in prep.) from the Council's January Sustainability Workshop, and the other from the NMFS technical ESA proceedings (Thompson 1991). These papers may offer some concrete

recommendations for assessing Type 1 risk. Along the same lines, a species definition paper has been written for the ESA technical process (Waples 1991); possibly aspects of this paper could be used to put "sideboards" on acceptable levels of several types of genetic risk.

ADAPTIVE MANAGEMENT / RESPONSE TO COUNCIL REVIEW

Genetic concerns played a large role in the Council's response (August decision memo) to the YKPP PDR in 1990. The two main genetic issues I raised in the GRA, Type 3 risks to Yakima summer steelhead from the small existing production program and to Yakima spring chinook from the proposed summer chinook reintroduction, were also of concern to the Council. The Council required specific actions to be taken to alleviate these sources of risk. The first, identification of steps to reduce impacts on steelhead from existing programs, has been satisfied by virtual elimination of the existing program. Steelhead production in the Yakima is now at a minimal level, producing only enough fish for the rainbow/trout interaction study. No new action has been taken on summer chinook except for dealing with it in the scaled-down options discussed below, but existing language in the PDR acknowledges the risk from the summer chinook program and considers identification of potential summer chinook impacts on spring chinook as a critical prefacility informational needs.

The Council's major expression of genetic concern over the project was a requirement that the genetic risk of various scaled-down versions of the Yakima program be assessed. This subject was discussed in depth at a March meeting of the Long-Term Fitness Team. Risks were assessed qualitatively for a number of options involving three types of reductions: 1) elimination of entire substocks; 2) elimination of specific substock/subbasin combinations; and 3) elimination of experimental groups. Results are summarized in Table 14. Not all possible reductions were discussed, but enough were to develop a pattern applicable to those options not explicitly considered. An approach rejected immediately was reduction of number of fish per group; group sizes are determined by experimental power requirements.

Table 14. Preliminary evaluation of genetic risks under various reduced-YKFP scenarios

Action	Type 1 Impacted Stock	Effect on Risk to Impacted Stock: Type 2 Extinction	Type 3 Within Pop. Diversity	Type 4 Among Pop. Diversity	Domest. Selection
Eliminate entire stock components from YKFP					
1. All (no YKFP)	All	varied	varied	varied	down
2. Summer chinook	Spring chinook	decrease	decrease	decrease	neutral
Eliminate subbasin/stock combinations					
1. Naches spring chinook	Naches springs	varied	varied	unknown	down
	American spring	down	down	down	N/A
	Yakima springs	varied	varied	down	neutral
2. Yakima spring chinook	Not considered a reasonable option.				
3. Yakima steelhead	Yakima sthd	varied	varied	varied	down
	Satus sthd	down	down	down	N/A
	Naches sthd	unknown	unknown	down	neutral
	Yakima rainbow	down/neutral	down/neutral	down	N/A
4. Naches steelhead	Not considered a reasonable option.				
Elimination of treatment groups					
1. Reduction to 9 spring chinook ponds in upper Yakima	Effects qualitatively same as elimination of entire group				
2. Reduction to 6 steelhead ponds in Naches	Effects qualitatively same as elimination of entire group				

Some of the entries in the table have been modified since the March meeting after further consideration, especially those pertaining to Type 1 and 2 risks ("varied" entries) in stocks not undergoing supplementation. The basic issue here is the "do nothing" option (for which the common entry is "varied"); our original opinion was that hatchery supplementation would decrease risks of all types except 4 because the stocks were in serious trouble as is. However, there were two assumptions here that could be debated. First, that the stocks are in serious trouble, and their long term persistence and identity is in jeopardy. This may be true, especially for small steelhead stocks that may occur in the Yakima, but we have not rigorously evaluated this for any stock in the project. The recently published list of 214 stocks determined to be at serious levels of extinction risk (Nehlsen et al. 1991) included Klickitat spring chinook, summer steelhead, and winter steelhead, but did not include any Yakima stocks. Thus, the viewpoint that all the YKPP stocks are in peril may not be very common. The second assumption implicit in the notion that the YKPP would involve less genetic risk is that the hatchery operation would work properly, that supplementation would actually result in more fish (and thus higher effective population size). Again, this is an assumption that could be easily debated. The recent review of supplementation by Miller et al. (1990) concluded that there is no strong evidence that supplementation really works (and also no strong evidence it doesn't). Testing supplementation is really the central experiment of the YKPP; we can't argue a priori that supplementation will be better for the stocks than doing nothing for them. It boils down to a question of relative risk.

A recent criticism of the project unrelated to the Council decision memo involves the genetic refuge stocks, American River spring chinook and Satus Creek steelhead. The 1990 GRA naively assumed that establishing genetic refugia was a simple matter. It is actually very complex. The American River situation illustrates this well. For this population to be protected from genetic risk, it has to be protected from straying from the enhanced Naches stock and protected from depletion due to mixed fishery harvests. An added complication is that this stock is not synchronized well with the Naches stock in terms of age at return. American River female spawners are virtually all five years old, whereas Naches females are a mix of fours and fives. An unsuccessful brood year will thus result in American River hitting a population low in different calendar years than the Naches. Harvest may have to be reduced on the Naches stock to protect the American River stock. The question of straying is another issue. How much straying is currently going on, and how good are our abilities to detect it? The argument could be made that the American River spring chinook may be at less risk if they are supplemented than if they aren't.' Obviously, genetic risk analysis is not complete without inclusion of a discussion of harvest policies and monitoring.

SUMMARY RECOMMENDATIONS

We should participate more actively in the formulation of the NPPC GRA guidelines to help bring that process to conclusion. Once the guidelines are in place, we should produce a revised GRA following those guidelines. This will still be an interim document, with a Level 2 approach possible for some stocks, but not others, but the revised GRA would be very useful. Internally, it will serve to delineate outstanding genetic risk issues and summarize our progress in resolving them. Externally, it will help the Council track our progress, reducing the chance of misunderstanding later.

The revised GRA should have extensive discussions on scaled-down production options and genetic monitoring. We may be at the point where the former is possible, but much developmental work is needed on the latter (see next section). An integral part of risk assessment is the ability to detect genetic impacts.

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GENETIC MONITORING ASPECTS OF THE YKFP

INTRODUCTION

More than any previous production project to be implemented, and more than any production program now planned, the YKFP stresses genetic conservation. Indeed, a key hypothesis to be tested in the YKFP is that new supplementation strategies can be used to increase production of salmon and steelhead in the Yakima and Klickitat subbasins without adversely affecting genetic resources existing in these drainages. There are two aspects to the testing of this hypothesis. The first is to ensure that the most theoretically and practically advanced procedures for minimizing genetic risk are used throughout the production program. The second aspect of testing this hypothesis is a genetic monitoring program (GMP) to ascertain the extent of genetic change occurring under the production program. In this section I discuss the options available for such a program, and some general issues concerned with developing it. This is largely a personal perspective, but my thinking has been heavily influenced by a chapter by Barrowclough and Lande ("Effective population size, genetic variation, and their use in population management") in Viable Populations for Conservation (M.E. Soule, ed.), by the comments of Supplementation TWG members (particularly Rich Carmichael) at a TWG meeting in July 1990, and a November MEG work session on genetic impact assessment.

Implicit in the following discussion is a central assumption that is essential for planning at this stage; that since the YKFP is intended to be a laboratory for supplementation and since minimizing genetic risk is central to the project, it follows that the YKFP should have the most comprehensive and technically sophisticated GMP possible. From this basic assumption follow three ancillary assumptions. The first is that we're interested in addressing all four categories of genetic risk outlined in the YKFP genetic risk assessment and currently used in MEG genetic impact assessment planning: 1) extinction, 2) loss of within-population genetic variability, 3) loss of population identity (among-population variability), and 4) domestication selection (which properly should include all types of anthropogenic directional genetic change, not just selection imposed by the hatchery environment). The second ancillary assumption is that logistical difficulties, including periodical decreased production capacity, are not a concern. The third assumption is that the price is no object.

Obviously, compromises will have to be made with logistical and financial reality in designing and implementing the GMP, and cost and logistical concerns will be addressed in discussing the various approaches that can be taken, but we need to be as idealistic as possible in initial planning. The YKFP is a model project, and the

GMP should be a model program as well. Planning for the GMP should be of lasting value not only for Columbia basin planning, but also for other basins and other species. In the long run, what we decide not to do is as important as what we decide to do, so it's important to carefully document our thinking in developing the GMP, and above all, to not discard ideas casually, no matter how expensive, difficult, or otherwise irrational they may seem.

TECHNICAL APPROACHES

Type 1 Impacts: Movement Toward Extinction

Extinction has important genetic consequences: once a population goes extinct, all its genetic material is lost. Type 1 impacts differ substantially from the other types of genetic impact we are trying to evaluate, however, in that the impacts themselves can quite often not be genetic. This may seem paradoxical, but demographic and environmental factors are generally much more important, especially at the early stages, in the extinction process. Only when the population size becomes small enough for serious inbreeding to take place (quantified by Barrowclough and Lande (1987) as "a few dozen", higher if the population has contracted rapidly from a large size) does genetics become important. Inbreeding depression will exacerbate the demographic problems, hastening extinction; this is termed an extinction vortex by Gilpin and Soule (1986). Minimum viable population size is often thought to be synonymous with minimum acceptable effective population size for avoidance of serious inbreeding, but the actual minimum viable population size for avoidance of extinction can be much larger.

Extinction theory today is a complex field. One basic idea is that there is no set minimum viable population size for any population. To set a minimum viable population size, survival of the population has to be stated in probabilistic terms, with a temporal component. Thus, rather than asking what would be the minimum viable population size, we should ask what is the minimum population size that will give us an 80% chance of survival for the next 200 years, for example. Another basic idea that I have found useful, is the concept of three extinction functions (Shaffer, 1987): demographic, environmental, and catastrophic. A fairly small increase in population size may yield a large benefit in persistence time via the demographic function, but enormous increases in population size may do very little to increase persistence time via the catastrophic function. The relationship between persistence time and population size on the environmental function is essentially linear. Population vulnerability analysis (PVA), developed by Gilpin and Soule (1986) links environmental and demographic stochasticity, metapopulation structure, and genetics to describe the process of extinction as a series of feedback loops.

Although the theory of extinction appears well developed, practical applications of it are scarce. **Soule's** (1987) book, Minimum Viable **Populations** for Conservation, has several chapters on extinction, some attempting to do something with the theory, but it is doubtful any of this can be of direct use to us. For example, we have no idea how to describe **Shaffer's** functions for anadromous salmonids. Larry Riggs is investigating if any of the theory can be used at this point, but at present, the Type 1 Risk module of the expert system he is designing for MEG bases the relative risk of extinction on intuitive reactions of a few geneticists to escapement data.

The upshot of all the above discussion is that the tools just haven't been developed to evaluate Type 1 impacts, so it is anyone's guess at this point how good a job we can do, beyond the point of stating that the population is at increased risk when the size gets small enough to cause inbreeding problems, information that will come from our monitoring of Type 2 impacts. Some good ideas came out the MEG work session, however. Escapement data are obviously important. A trend of escapement decline can be taken as movement toward extinction, and possibly so can wildly fluctuating escapements. Demographic data such as age structure, sex ratio, and fecundity are also very important; to have any hope of ever applying a sophisticated demographic model to our populations, we obviously need to have some understanding of the actual demographics of the populations. Age structure data will also alert us to the problem of year class failure, which will increase extinction risk. Management information can give us insight into Type 1 risk and impact as well. Escapement goals, management to achieve escapement goals, and threshold levels to trigger fisheries are all important, since harvest can be a large component of the catastrophic extinction function.

Type 2 impacts: loss of within-population variability

Type 2 impacts can be evaluated far more accurately and simply than impacts of any other type. All that is required is that we monitor within-population genetic variability. This can easily be done with electrophoresis. We can estimate single-gene heterozygosity readily and monitor this over time. We can also estimate effective population size with electrophoretic data (Waples 1989, 1991). Although protein electrophoresis is the most widespread technique for looking at heterozygosity, **it's** not the only one. Genetic variability can be estimated from a variety of characters: nucleic acid sequences, chromosomal polymorphisms, and simple visible Mendelian polymorphisms.

Evolutionarily, additive genetic variation at quantitative traits is far more important than single-gene variability. Monitoring variability of this sort is more complicated because expression at quantitative traits (such as fecundity, body size, migration

timing, other behaviors, etc.) is a mix of genetic and environmental effects. The standard method is to estimate the heritability of the trait, the ratio of additive genetic variance to total phenotypic variance. There are a variety of ways to do this, but the simplest method is to measure the trait of interest in parents and their offspring and regress offspring performance on parental performance for the trait. Choice of trait is important, because heritabilities and their standard errors are inversely correlated; the larger the heritability, the lower the standard error. Since our intent will be to monitor overall changes in genetic variability rather than a specific trait, the simplest approach is to periodically estimate the heritability of a high heritability trait (heritabilities have been estimated for a variety of traits in cultured salmon and trout).

Recently fluctuating asymmetry analysis has been used to evaluate differences in genetic variability. The theory is that decreased variability causes developmental instability, and this in turn causes meristic counts on the two sides of a fish to differ. There is still some question about its ability to detect other than gross changes in variability, but recent work by **Allendorf's** group has shown the technique to be fairly sensitive to variability changes in trout (e.g., Leary et al. 1985). Another concern, based on anecdotal reports of work by Winans, is that increased asymmetry can be caused by outbreeding depression, a Type 3 impact. The technique is very simple. Meristic characters are counted on both sides of a fish, and the differences between the two counts calculated.

To this point I've only discussed simple loss of overall variability. Another subcategory of Type 2 impact is loss of life history variability due to nonrepresentative broodstock selection. The only way I see to evaluate this sort of impact is to monitor the trait in the population, watching for changes.

Evaluation of Type 2 impacts poses some practical problems. The primary tool, starch-gel electrophoresis may not work in some species, because of lack of electrophoretic variability. This is not a problem in chinook and steelhead, but is in **coho.** Otherwise electrophoretic evaluation of Type 2 impacts is straightforward. Heritability estimates will pose substantial logistical difficulties, however, because families of fish will have to be reared separately. If the trait to be analyzed is measured on adults, smolts will have to be tagged with family codes. Fluctuating asymmetry analysis requires no special hatchery design features or disruption of normal operations.

Type 3 Impacts: Loss of Amons-Pooulation Variability (Population Identity)

This type of impact is caused by population mixing, so to monitor this impact we need to look for changes in frequencies of electrophoretically detectable alleles, nucleic acid restriction fragment length polymorphisms (RFLP's), chromosomal polymorphisms, visible Mendelian alleles, or shifts in mean of quantitative traits. When sufficient electrophoretic variation is present, as is the case with chinook and steelhead in the YKFP drainages, the electrophoretic approach is probably the most feasible. Not only can allele frequency shifts at single loci be monitored to give estimates of gene flow, but the shifts can be summarized as genetic distance statistics, so estimates can also be made from changes in these distances. There is a limit to the sensitivity of these analyses, however, so information on straying rates is essential. We should have baseline data on presupplementation straying rates and then monitor changes as supplementation proceeds. To do this with any sensitivity, we need a fairly large tagging program.

Monitoring for Type 3 impacts present only one theoretical difficulty, so long as measurable genetic differences exist between the stocks involved: being able to distinguish true Type 3 impacts from natural genetic change. Evolution is most simply defined as allele frequency change, so we will expect allele frequencies to change over time anyway. Corroborating straying information is vital in this case. Another aspect of this problem is allele frequencies shifting due to genetic drift. This happens in all finite populations; the amount of the shift is a function of the effective population size (N,). We plan to have several years' electrophoretic information before supplementation begins, and we can estimate N_e from these data using Waples' method. This can cause a circularity of argument, however, if allele frequencies are fluctuating and we try to explain the fluctuation by an estimate of N_e that is itself based on the fluctuating frequencies. An independent estimate of N_e would be helpful, and this can be gotten only from demographic data (discussed below in Core Data section).

Monitoring for Type 3 impacts can largely be done within normal operations, so this presents no practical problems in implementation. Tagging fish with stock-specific codes for straying rate estimation should not interfere with normal operations. Collecting some of the demographic data may impose quite a load, however, in that families may have to reared individually and tagged individually (see Core Data section).

Type 4 **Impacts:** Domestication Selection

Considerable controversy exists over the magnitude of Type 4 impacts, especially those caused by hatchery rearing. While probably all geneticists will admit that hatcheries impose selective effects much different from those encountered in the natural environment, they differ widely in their opinions of how serious the impact is. Conservation biologists typically deal with emergency situations, such as an entire species going extinct or entire ecosystem disappearing. They often have to include zoo animals as part of their breeding population. Many of them would consider worrying about the genetic impacts of captive propagation either as immaterial or as a luxury. Little solid experimental work has been done on Type 4 impacts, so hard data are in short supply. The best studies on Type 4 impacts are Reisenbichler and McIntyre **(1977),** Chilcote et al. **(1986),** and Swain and **Riddell** (1990).

Type 4 impacts are considerably more difficult to evaluate within a production scheme than other types of genetic impacts because there is little likelihood that they will be reflected in genetic characteristics that are simple and inexpensive to measure, such as electrophoretic variation. The impacts will almost certainly only be expressed as performance differences at quantitative traits. To evaluate Type 4 impacts then, we will need to compare performance at a quantitative trait or suite of traits with and without the effects of selection. Five approaches are possible (if you can think of others, let me know):

1. **Pre-** and **Post-** Monitoring. This is the simplest of the four, involving no experimental setup, just data collection. The population or populations of interest are monitored intensively for the trait(s) for as long as possible before the production program begins to establish a performance baseline, and then the monitoring is during the production program. Ideally monitoring will be continuous, but this isn't essential. Data collected from the supplemented population can be compared at any time with the pre-supplementation data or with earlier supplementation data to give an estimate of Type 4 impact.

   This approach's strength is simplicity; it would be the easiest of the four alternatives presented to accomplish. It has a very serious weakness in that there is no way to separate genetic and environmental effects. I see no way around this problem at this point.

2. Control and Treatment **Monitoring** This is a straightforward experimental approach. Some populations are supplemented (receive the experimental treatment) and some are not (serve as controls). Ideally this is done with a series of paired populations, each pair composed of similar streams with genetically similar populations. The more pairs, the stronger the statistical power. Traits of interest are monitored in all

populations, and performance of control and supplemented populations are compared. A variation of this is to split a population in a single stream, supplementing half and not supplementing the other half. The two groups would have to be marked, and kept separate. This design.has advantages over the basic design in that the environmental variation due to stream differences is eliminated.

There are problems in attributing whatever difference is found to Type 4 impacts, however. In any control-supplementation population pair, both populations will change over time due to genetic drift. This is a random process, so they may become more similar, or more distinct, and all this is separate from the actual Type 4 impact. This makes it even more important to have as many population pairs as possible. A second problem is that the control and treatment groups should be independent. Within a single production project, it is difficult to imagine this happening, the increased numbers of the supplemented stock may mean increased straying into the control stock, thus changing its genetic character. Supplementation and control juveniles may also interact ecologically. This will be minimized by use of smolts, but the possibility can't be ruled out.

The theoretical problems just mentioned assumes that the major obstacle in implementing this type of study has been solved, that of finding the population pairs for the research. In practice, within the confines of a single production project, these pairs will likely be impossible to find. I see no possibility for coming up with a convincing experimental design for spring chinook in either subbasin. Yakima steelhead are a possibility, but we don't know enough about their substock structure yet. The refuge populations, American River chinook and Satus Creek steelhead, are at best quasi-controls, since they are genetically distinct (especially American River) from other populations in the subbasin, and they occupy habitats which are dissimilar to any others in the subbasin (here again, more true of American River). The quasi-control approach has possibilities, but to work needs a great amount of replication. This could be done as a coordinated effort over several projects, or in a project spanning several subbasins such as the Northeast Oregon project.

The alternate design, using supplemented and unsupplemented populations in the same stream, has a very serious drawback: the need for keeping the subpopulations separate during spawning. Hatchery fish have to be kept out of the spawning grounds, which would require a weir below all possible spawning habitat. This design is obviously unworkable in the YXFP, because it is planned to use exclusively non-hatchery fish for broodstock.

3. Single-Generation Genetic Change. In this approach, at any

particular point desired after supplementation begins, hatchery x hatchery (HH) and **"wild"** x **"wild"** (WW) crosses are made in the hatchery. Eggs and fry are treated identically. Performance differences between the two groups can be measured at any point during the life cycle, before release or after return as adults. If desired, hatchery x **"wild"** crosses can also be made. Since the rearing environment is identical for all groups, any differences between groups will be genetic.

The major theoretical problem with this approach is that differences in performance between groups can be counted on to reflect only one generation of Type 4 impacts; we know **"wild"** fish were not raised in the hatchery, but their parents may well have been. Not knowing what magnitude a single generation impact will have, it is unclear how large the experiment will have to be. A second problem is that the approach only gives us half of the impact; i.e. how well **"wild"** fish perform in the hatchery. We're very interested in how hatchery fish perform in the wild, especially in spawning.

This approach presents practical problems for the YKFP. Production of HH fish is not part of the overall program; all production supplementation fish are planned to be the progeny of **"wild"** parents. So either the HH progeny have to be considered as an experimental group and destroyed upon return to the subbasin, with the consequent temporary loss of production, or the occasional production of HH progeny has to be accepted as the cost of evaluating Type 4 impacts. In the YKFP all hatchery fish will be marked. If test groups are to be released for evaluation upon return, they must receive an additional mark to allow them to be separated from normal production fish.

4. Genetic Marking of Test Groups. Genetic marking has great appeal for studies of Type 4 effects, as the fish are internally marked and a project can be designed where their progeny can be marked as well. It's difficult to generalize about this approach, but examples illustrate the approach quite well. Reisenbichler and McIntyre (1977) made matings of hatchery and wild fish to generate HH, HW, and WW progeny of particular genotypes, planted the offspring in closed stream test sections. After a few months they collected fish from the test sections, and electrophoresed them to determine the relative survival of the three genotypes and thus the Type 4 impact. Chilcote et al. (1986) raised the frequency of a rare allele to high levels in a hatchery steelhead stock by selective mating of pre-genotyped adults, then stocked the fish into the Kalama River, where the allele was in low frequency. Success of the hatchery stock was monitored by increases in the frequency of the marker allele. Phelps and Busack proposed a study of relative reproductive success of hatchery and wild adults in the Tucannon River. Adults were to be biopsied upon

collection at the weir and equal numbers of hatchery and wild fish of appropriate genotype (H were all one genotype, W another) were to be let into a test section of stream to spawn. Relative success could be judged by sampling alevins, fry, or outmigrating smolts by comparing the allele frequency in offspring to that expected form the mix of adults. Numerous other designs can easily be imagined.

Theoretically the biggest problem with genetic marking studies is that the population must be manipulated genetically to study it. When a rare allele is raised to a high frequency by selective mating of only those fish carrying the allele, it is likely that relatives are being mated, so the progeny will be inbred (the inbreeding coefficient can probably be calculated fairly easily). Using inbred marked fish can easily bias the results of a study to estimate Type 4 effects, because the marked fish will be expected to be less fit than the unmarked. This can be avoided using passive rather than active genetic marking, using existing fish rather than selectively breeding fish to mark them. **Phelps'** design is a case in point. The passive marking technique has much lower statistical power than the active, however. A second aspect of this problem is the ultimate genetic change caused in the population by the study. It is difficult to avoid manipulating the population allele frequencies, but allele frequency change can be minimized by using the passive technique with a marker allele at an appropriate frequency.

It is as hard to generalize about practical problems encountered in implementing genetic marking studies in the YKFP as it is to generalize about their design, but genetic marking studies can easily involve stream test sections, perhaps limiting production opportunities and reducing natural spawning or rearing habitat. If a marker strain is to be developed, this will require allocation of hatchery resources over a considerable period. As mentioned above, genetic marking can easily result in allele frequency change, and this will likely be hard to reconcile with YKFP goals of minimizing genetic change.

5. Direct Measurement of Genetic Trend. This is the most experimental, highest-tech approach, but also the one with the most promise. Sperm from several males is cryopreserved before supplementation begins. At any desired time thereafter, the eggs from one group of females will be fertilized by sperm from contemporaneous males and the eggs from another group of females will be fertilized with cryopreserved sperm. The difference in performance between the two groups will be solely genetic. For increased experimental power, the eggs of each female should be incubated separately and the resulting progeny reared separately. If they are to be released for evaluation upon return, the smolts should receive family-specific tags.

For an even more powerful design, only one group of females would be used, but the eggs of each would be split into two lots, one to be fertilized by a contemporaneous male and one to be fertilized by cryopreserved sperm. This approach is the only one discussed here that would allow genetic changes over multiple generations to be evaluated easily.

The only theoretical drawback to this approach is fairly minor: the cryopreservation of sperm only will not allow expression of presupplementation mitochondrial DNA. The analysis will thus be a comparison of nuclear DNA of presupplementation and supplementation fish in a mitochondrial background of supplementation fish only. If there has been a substantial change in the mitochondrial genes due to domestication selection, this won't be detected. Thus, the impact will be under estimated. I don't know what the impact of selection should be on mitochondrial genes.

Some of the practical problems have already been mentioned. Sperm cryopreservation in salmonids is still relatively new, and therefore somewhat chancy. Gary Thorgaard at WSU has made substantial improvements in cryopreservation techniques, and now feels the method is ready to apply to situations like this. Aside from the technique's newness and potential riskiness, the only other practical problems are the possible need for separate-family rearing, and the loss of production capability that devoting hatchery space to this analysis may incur.

Regardless of which approach to Type 4 impact analysis is taken, choice of traits to be evaluated is critical. Traits must first and foremost be relevant to production and conservation goals; reproductive traits are obvious choices. They should also be relatively insensitive to environmental influences; the more environmental noise encountered, the harder it will be to find the genetic impact. Another consideration is variance; we'll be looking for changes in mean, so low variance will provide higher statistical power. Finally, the traits should be relatively simple and inexpensive to measure.

OTHER CONSIDERATIONS

Separating Anthronoaenic Impacts from Evolution

I spent some time on this in discussing Type 3 impacts, but should have addressed it throughout. Our ability to discriminate between natural and supplementation caused changes is limited, especially with some proposed designs. When we're looking at options, we need to consider them with this in mind, For example, the cryopreservation approach to measuring genetic trend is extremely powerful for measuring genetic change over time, but you can't

unequivocally say the changes you've measured are caused solely by supplementation. With the single-generation approach, you can be much more confident (although not 100%).

Sample Sizes, Sampling Frequency, and Statistical Power

In the discussion above I haven't mentioned any specifics about how much, how often, and how powerful our analysis will be. I have some ideas about particular items, but no generalization is possible at this point. We need to narrow the options down as much as we can (do the best job possible of answering what) then explore these aspects of the monitoring program. In most cases these questions can be answered **quite** easily, and we can start developing cost estimates.

Core Data

At the MEG work session on genetic impact assessment the concept of core data was discussed. The basic idea is that there are certain data that should be collected in all production efforts, data that will aid both production monitoring and genetic monitoring. We talked specifically about demographic data such as age structure, sex ratio, escapement, fecundity, and variance of family size. Careful monitoring of these would incur a logistical and financial load, but good estimates of these parameters are important for estimates of effective population size, which would aid in assessing Type 2 and 3 impacts, and for serious work on Type 1 impact, as well as generally increasing the overall ecological understanding of our populations. I think it's difficult to argue that increasing one's understanding of the demographics of a population is not useful.

Hatchery Modifications

It may be desirable to have the capability of rearing families of fish individually. Current hatchery plans for the YKFP don't allow for this, so modifications will be needed if this capability is required. One application of this capability is estimation of family size variance. This is the one key component of effective population size that is virtually always missing from N_e calculations, and it can have a profound effect. Somewhere in the Columbia basin someone should try to estimate effective population size carefully, and if not in the YKFP, then where?

Individual rearing of families will also make heritability estimates possible, desirable for assessment of Type 2 risk. Undoubtedly other needs for individual family rearing will arise as work proceeds; for this to be a good experimental facility where

serious genetic research can be done, we need to be able to rear fish this way.

Overall and Specific Monitoring

Recent discussions within the Joint Reproductive Success/Long Term Fitness Team have revealed a perceptual distinction regarding the GMP. There are actually two needs to be fulfilled by the GMP: 1) an overall need to monitor genetic change, assuming the entire YKFP is the experiment (i.e, to answer the question of how little genetic change can we incur and still have a state-of-the-art supplementation program?); and 2) specific needs to answer the question of what genetic changes are brought about by particular supplementation experiments. The methodologies will probably not differ substantially, but experimental power will differ markedly in the two approaches.

SUMMARY RECOMMENDATIONS

The distinction between overall and specific monitoring needs must be clearly delineated by the LTF and RS teams before any further progress can be made in development of the GMP, so this should be a major topic of discussion for the teams at the earliest opportunity. Once this matter is settled, work can begin on a draft GMP.

The first step in the development of the draft GMP should be for the LTF and possibly RS teams to take an initial cut at the options available for monitoring each type of impact, resulting in a GMP skeleton. WDF will do much of this, but there will have to be a substantial amount of full team participation on the subject of quantitative variation, specifically regarding life-history and morphological response variables. The core-data concept will also have to be more fully discussed. We expect that Michael Lynch, the YKFP genetics consultant, will be quite active participant in development of the GMP, beginning with this stage.

Once the skeleton GMP is developed, an analysis of experimental power will be done to show what level of genetic impact can be measured at what effort and cost. Just as a minimum viable population size has to be defined in terms of probability of population persistence over a given period of time, the monitoring program power will have to be defined in terms of probability of detecting a specified percentage change over a specified time period. The evaluation of power will probably have to be done using a stochastic model.

After the power calculations are done, the cost and effort of monitoring change at specified levels will be assessed. As already stated in the background document, design of the YKFP GMP should

not be initially limited by cost. In developing a comprehensive GMP we are plowing new ground; it is vital for both this effort and future monitoring efforts to do a thorough analysis of how much a given level of monitoring will cost.

The resulting draft GMP should be widely circulated for review to interested geneticists, preferably as part of the next revision of the genetic risk assessment. A possible (and desirable) byproduct of the development process is a genetic monitoring manual.

Work should begin now in earnest to develop the GMP draft. It is needed in the short run for further revision of the genetic risk assessment. In terms of long-term consideration, development of the final GMP will be a lengthy process, so we need to get this initial draft developed as soon as possible.

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SCALE PATTERN AND AGE/LENGTH ANALYSIS OF 1989 AND 1990 YAKIMA RIVER ADULT SPRING CHINOOK

INTRODUCTION

The following section describes age and growth analyses of 1989 and 1990 returning adult Yakima River spring and fall chinook salmon *Oncorhynchus* tshawytscha related to the prefacility phase of the YKFP. The pre-implementation process requires the identification and characterization of existing substocks in terms of genetics, life history traits, growth, and age.

The purpose of this portion of the study is to describe the characteristics of adult Yakima River spring and fall chinook collected in 1989 and 1990 based on age, length, and sex and to determine if scale pattern analysis can be used to characterize and separate naturally rearing groups of spring chinook.

METHODS

Sample Collection

Scale samples were collected from snagged live fish or carcasses recovered on the spawning grounds for genetic stock identification (GSI) analysis by Washington Department of Fisheries (WDF) personnel. Nine areas within the Yakima River were sampled representing seven groups of spring chinook and two groups of fall chinook: American River, Bumping River, Naches River, Little Naches River, Yakima River (Easton), Cle Elum River, and Yakima River (below Roza). Six scales per fish were collected from the International North Pacific Fisheries Commission preferred body area (Major et al. 1972) and mounted, at the time of collection, on gummed scale cards. Post-orbital to hypural plate (POHP) lengths to the nearest cm and sex were also recorded. Acetate impressions of the scales were made (Clutter and Whitesel 1956) and subsequently used in ageing and measuring scale patterns.

Aae Analysis

A total of 505 fish were collected in 1989 and 348 in 1990. Scales were aged by two WDF scale analysts using a micro-fiche reader at 24 and 48 X. Unaged scale samples were either regenerated, obscured by dirt, missing, or mounted upside down. When age determinations for a fish differed between analysts the fish was reaged by both analysts and a consensus reached. The European form of age designation is used for adult ages in this report (Koo 1962). For example, age 1.3 designates that one complete winter was spent in freshwater after hatching (the numeral to the left of the decimal

point) and 3 complete winters were spent in the ocean (the numeral to the right of the decimal point) and the fish is in its fifth total year of growth. The Gilbert/Rich form of age designation would represent an age 1.3 fish as 5_2 and an age 0.3 fish as 4_1. Juvenile fish which have not completed one full year in freshwater are designated age 0+ and those that have completed one year are designated age 1+ (yearlings).

Scale Pattern Measurements

A microcomputer/video-camera based digitizing system was used to measure scale patterns. The distance from the center of the focus to the outer edge of the first circulus (the dark concentric bands on scales) and the intercirculus distances between the first 34 circuli were measured to the nearest 3.2 microns (the width of a digital sampling unit) along a measurement axis 90° (±1 degree) to a reference line (Fig.6). The reference line is constructed by connecting the two ends of the first marine annulus in the area where the posterior and anterior portions of the scale meet. Intercirculus measurements were converted via a FORTRAN program to 11 scale character variables by combining consecutive sets of three intercirculus distances into "triplets" (Table 15).

Table 15. Scale character variables used in scale pattern analysis for group discrimination. All variables are distance measurements in microns.

Variable name	Definition
Focus	The distance from the center of the focus to the outer edge of the first circulus.
T1	The first intercirculus triplet beginning at the outer edge of the first circulus and extending out to the outer edge of the fourth circulus.
T2, T3, ..., T11	The distance from the outer edge of circulus ((3*i)-2) to the outer edge of circulus ((3*i)+1), where i equals 2, 3,..., 11 and i represents the triplet of interest.

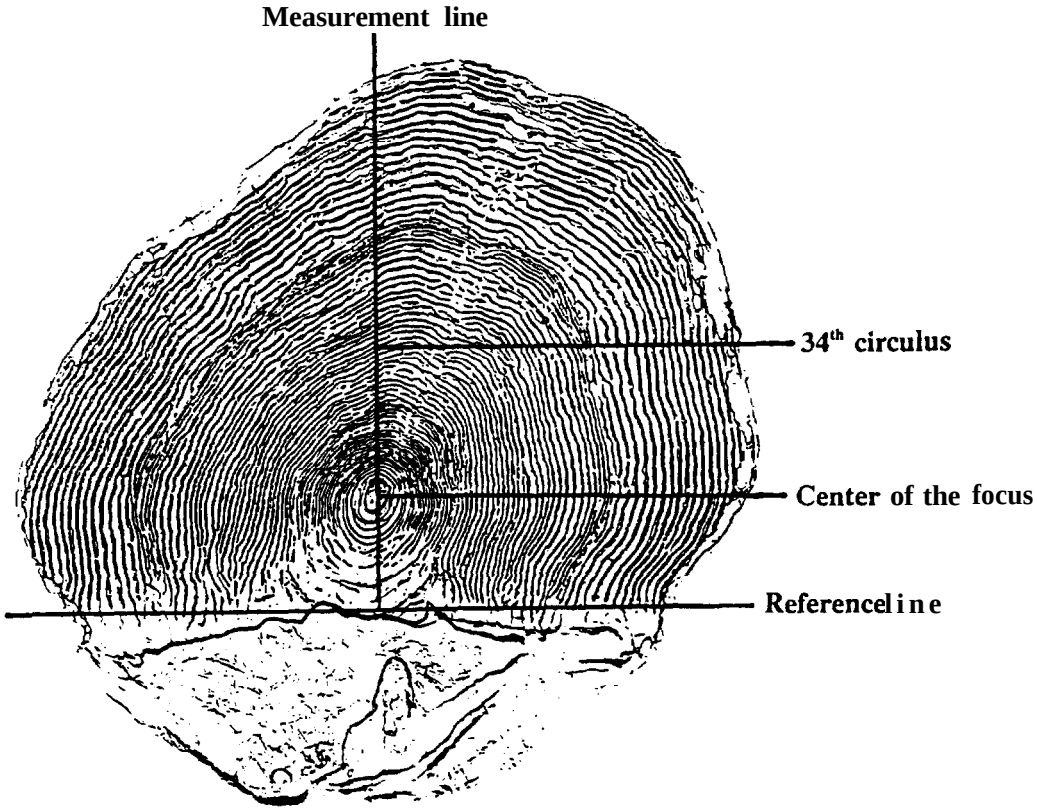

Fig.6. An adult 1986 Skykomish Hatchery coho salmon scale. showing the measurement line, reference line, center of the focus and the 34th circulus.

Linear Discriminant Function and Canonical Variates Analysis

Two statistical methods were used to examine scale measurements and identify groups of spring chinook possessing distinct scale patterns: linear discriminant function (LDF) analysis and canonical variates (CV) analysis. The BMDP 7M discriminant analysis software was used to perform the LDF and CV analyses (Jennrich and Sampson 1988). When the basic assumptions of **equal** covariance structure and multi-variate normality are meet, LDF and CV analysis give identical classification results (Williams 1983). In CV analysis, the scale variables entered into the first linear discriminant function are weighted so that among-group variation is maximized relative to within-group variation (Lachenbruch and Goldstein 1979). The coefficients for another set of **CV's** are calculated along an axis orthoginal (at a **90°** angle) to the first axis and the scale variables are again weighted so that group differences are maximized along this second axis. This procedure is repeated for each successive canonical variate until there are up to g-1 or p **CV's,** whichever is smaller, where g is the number of groups and p is the number of scale variables. The first two canonical variates typically explain 70 percent or more of the total variation in the model. Knudsen (in press) used two-dimensional plots of the first two **CV** to geometrically describe the relationships of groups of **coho** salmon. Groups separated by large distances have dissimilar scale patterns, indicating growth rates differed significantly, while closely spaced groups have similar scale patterns and cannot be accurately separated. In each LDF and CV analysis below, the 12 scale variables were forced into the discriminant functions. That is, no variable selection procedure was used.

CV's are standardized along each orthogonal axis so that the overall mean is 0 and pooled within-groups standard deviation is 1. This is comparable to standardizing to the z-distribution in the univariate case by subtracting the mean and dividing by the standard deviation. Thus, distances along each axis in a CV plot are dimensioned in terms of the pooled within-groups standard deviation.

General Simulation Methods

Simulation analyses were performed using a FORTRAN program written by Millar (1988) and configured for use on **WDF's** PRIME computer by J. Packer, WDF. The program creates simulated mixture and standard samples and estimates mixing proportions using LDF analysis with error correction. A classification rule consisting of linear functions based on standard samples is used to calculate the likelihood of observing a fish's measurements if that fish was from group i, i= **1,...,** number of groups (Lachenbruch 1975). The fish is then classified into the group for which the likelihood is highest. The program uses the apparent error rate matrix rather than the

jackknifed error matrix (Lachenbruch and Mickey 1969) for correcting classification results for bias in the classification rule that occurs when estimates of group composition are made (Cook and Lord 1978). When standard sizes are large enough **(≥100)** the difference between the apparent and jackknifed error rate estimates will be small (Millar 1988). In order to ensure that corrected composition estimates are constrained between 0 and 1, the method of Cook (1983) is used.

The simulation analyses attempt to reproduce the randomness involved in practical sampling applications. A set of measurements from known origin standards is required. To simulate new standards and mixed samples, the given standards are sampled with replacement. Thus, to simulate creation of a 200 fish standard from group i, the group i standard is sampled 200 times with replacement. Mixture samples are created by randomly varying each standard's proportion in the mixture. That is, for any given mixture sample the known proportion of stock i can vary from 0 to 1 (determined by a random number generator) and the required number of fish are then sampled from stock i with replacement. Each simulation involved estimating a mixture's composition using bootstrapped standards and this process was repeated 400 times for each analysis. **Millar's** program was modified to calculate the error in each estimate, defined as the difference between the known and estimated proportion of each group in the mixture. The mean of the errors is a measure of overall bias of the model and the standard deviation of errors is a measure of the model's precision.

Table 16. Number of fish aged and digitized, percent age composition, and mean length (POHP) and standard deviations for 1989 and 1990 Yakima River spring chinook by group, age, and sex. Mean age of female reproduction for each group is given, as well.

Race	Group	Year	Age	Sex	Number aged	Percent	Number digitized	Mean POHP length - cm	Mean age of female reproduction
Spring	American River	1989	1.1	male	0	0	0	---	3.9
				female	0	0	0	---	
			1.2	male	27	33.3	20	59 (5.5)	
				female	3	3.7	1	59 (4.4)	
			1.3	male	21	25.9	17	76 (5.9)	
				female	30	37.0	20	73 (3.1)	
		1990	1.1	male	1	1.1	0	40 (0.0)	3.9
				female	0	0	0	---	
			1.2	male	12	13.5	0	61 (6.4)	
				female	7	7.9	0	61 (2.4)	
			1.3	male	24	27.0	0	74 (6.1)	
				female	44	49.4	0	73 (3.2)	
			1.4	male	1	1.1	0	75 (0.0)	
				female	0	0	0	---	
Spring	Bumping River	1989	1.1	male	0	0	0	---	3.7
				female	0	0	0	---	
			1.2	male	7	31.8	5	59 (3.8)	
				female	4	18.2	3	60 (1.3)	
			1.3	male	3	13.6	1	68 (2.6)	
				female	8	36.4	2	73 (4.7)	
		1990	1.1	male	0	0	0	---	3.5
				female	0	0	0	---	
			1.2	male	8	20.0	0	59 (9.2)	
				female	10	33.3	0	60 (4.5)	
			1.3	male	4	13.3	0	74 (4.7)	
				female	10	33.3	0	69 (3.2)	
Spring	Naches River	1989	1.1	male	3	4.1	1	46 (9.6)	3.7
				female	0	0	0	---	
			1.2	male	28	37.8	20	57 (4.1)	
				female	10	13.5	10	63 (2.6)	
			1.3	male	8	10.8	7	75 (4.5)	
				female	25	33.8	17	70 (4.2)	
		1990	1.1	male	0	0	0	---	3.3
				female	0	0	0	---	
			1.2	male	25	41.7	0	57 (5.4)	
				female	19	31.7	0	61 (3.9)	
			1.3	male	8	13.3	0	73 (4.5)	
				female	8	13.3	0	73 (4.1)	
Spring	Little Naches	1989	1.1	male	0	0	0	---	3.7
				female	0	0	0	---	
			1.2	male	11	28.2	9	56 (5.1)	
				female	8	15.4	4	61 (1.5)	
			1.3	male	8	15.4	5	75 (5.6)	
				female	18	41.0	13	71 (3.2)	
		1990	1.1	male	0	0	0	---	3.8
				female	0	0	0	---	
			1.2	male	10	50.0	0	57 (3.8)	
				female	2	10.0	0	60 (0.0)	
			1.3	male	2	10.0	0	67 (4.9)	
				female	6	30.0	0	67 (5.4)	

Table 16. (cont.)

Race	Group	Year	Age	Sex	Number aged	Percent	Number digitized	Mean POHP length - cm	Mean age of female reproduction
Spring	Yakima R. Easton	1989	1.1	male	3	3.1	2	42 (5.2)	3.1
				female	0	0	0	---	
			1.2	male	33	34.0	18	55 (3.8)	
				female	53	54.6	27	57 (3.3)	
			1.3	male	2	2.1	1	73 (1.4)	
				female	6	6.2	5	66 (4.1)	
Spring	Cle Elum River	1989	1.1	male	0	0	0	---	3.0
				female	0	0	0	---	
			1.2	male	32	33.7	13	58 (3.6)	
				female	59	62.1	36	56 (3.0)	
			1.3	male	2	2.1	0	73 (3.5)	
				female	2	2.1	1	65 (2.1)	
Spring	Yakima R. (Easton and Cle Elum)	1990	1.1	male	0	0	0	---	3.0
				female	0	0	0	---	
			1.2	male	15	32.6	0	57 (4.5)	
				female	31	67.4	0	55 (3.0)	
			1.3	male	0	0	0	---	
				female	0	0	0	---	
Spring	Yakima below Roza	1989	1.1	male	0	0	0	---	3.0
				female	0	0	0	---	
			1.2	male	1	0.3	0	56 (0)	
				female	11	91.7	9	56 (2.0)	
			1.3	male	0	0	0	---	
				female	0	0	0	---	
		1990	1.1	male	1	1.0	0	36 (0.0)	3.0
				female	0	0	0	---	
			1.2[a]	male	33	31.7	0	55 (3.4)	
				female	67	64.4	0	56 (2.9)	
			1.3	male	1	1.0	0	64 (0.0)	
				female	2	1.9	0	70 (0.7)	

[a] This excludes 5 fish which were not sexed.

RESULTS AND DISCUSSION

Chinook salmon age compositions by race (spring and fall), group and sex are given in Tables 16 and 17. Mean POHP length and the number of fish digitized for scale pattern analysis are also given in Tables 16 and 17. All adult spring chinook recoveries migrated to the ocean as age **1+** smolts. The Naches system groups were primarily age 1.3 (mean 53 percent in 1989 and 47 percent in 1990) and age 1.2 (mean 45 percent in 1989 and 52 percent in 1990). Age 1.1 jacks were found only in the 1989 Naches River (4 percent), 1990 American River (1 percent), 1989 **Easton** (3 percent), and 1990 Below Roza (1 percent) samples. **Easton,** Cle Elum River, and below Roza spring chinook samples ranged from 89 to 100 percent age 1.2 in both 1989 and 1990.

Nearly all adult fall chinook samples migrated to the ocean as age 0+ smolts. Only one age 1.1 and one age 1.3 fish were recovered in the 1990 **Benton** City sample. Fall chinook ages varied considerably, but were primarily age 0.2 (range 7 to 74 percent) and age 0.3 (range 0 to 75 percent). The 1989 and 1990 Marion Drain samples were unique in that they contained 25 and 23 percent age 0.1 jacks, a much higher percentage of jacks than any other group of fall or spring chinook.

Significant differences **(P<0.001)** in length were found between age 1.2 and 1.3 1989 Naches system chinook in a two-way **ANOVA** of length by group (American, Little Naches, and Naches) and age (1.2 and 1.3). There were no significant group **(P>0.26)** or interaction effects **(P>0.13)** indicating that the lengths of age 1.2 and 1.3 fish were similar across groups. Thus POHP length may accurately predict age of 1.2 and 1.3 fish from the Naches system. Only groups with at least 10 fish per group/age cell were included in this analysis. There were insufficient samples to include groups outside the Naches system or to make a meaningful test of sex effects on length.

The accuracy of POHP length as a predictor of age of spring chinook from throughout the entire Yakima River system was then tested using LDF analysis. Lengths of 1989 spring chinook were pooled by age class to create an age 1.2 **(n=286)** and 1.3 **(n=129)** standard. An LDF analysis was then performed using these two standards with POHP length as the discriminating variable. This two-way age **1.2/1.3** model had an overall unweighted classification accuracy of 96 percent (age **1.2's** 97 and age **1.3's** 94 percent correctly identified). Computer simulations (see General Simulation Methods above) using these two standards had a mean error of less than 0.5 percent over a wide range of mixing proportions and approximately 95 percent of the age estimates fell within 7 percent of their true value. There were not sufficient numbers of age **1.1's** to establish a statistically meaningful standard and include them in this analysis.

Table 17. Number of 1989 and 1990 Yakima River fall chinook aged and digitized, percent age composition, and mean length (POHP) and standard deviations by group, age, and sex. Mean age of female reproduction for each group is given, as well.

Race	Grow	Year	Ag	Sex	Number axed	Percent	Number digitized	Mean POHP length - cm	Mean female age of reoroductfon
Fall	Benton city	1989	0.:	male	0	0	0	---	2.5
				female	0	0	0		
			0.2	male	0	0	0	---	
				female	3	50.0	0	55 (1.2)	
			0.3	male	2	33.3	0	81 (4.9)	
				female	0	0	0	---	
			0.4	male	0	0	0		
				female	1	16.7	0	73 (0)	
		1990	0.1	male	7	6.6	0	39 (2.4)	3.0
				female	0	0	0	---	
			0.2	male	5	4.9	0	55 (4.7)	
				female	3	2.9	0	60 (8.7)	
			1.1	male	1	1.0	0	48 (0.0)	
				female	0	0	0	---	
			0.3	male	26	25.2	0	71 (5.6)	
				female	51	49.5	0	70 (5.0)	
			1.3	male	1	1.0	0	71 (0.0)	
				female	0	0	0	---	
			0.4	male	5	4.9	0	79 (2.2)	
				female	S	4.9	0	75 (1.9)	
Fall	Marion Drain	1969	0.1	male	21'	24.7	0	41 (3.1)	2.0
				female	0	0	0	---	
			0.2[b]	male	38[a]	44.7	0	52 (3.5)	
				female	25[a]	29.4	0	57 (4.6)	
			0.3	male	0	0	0	---	
				female	0'	0	0	---	
			0.4	male	1	1.2	0	78 (0)	
				female	0	0	0	---	
		1990	0.1	male	14	23.3	0	41 (3.2)	2.7
				female	0	0	0	---	
			0.2	male	20	33.3	0	53 (4.1)	
				female	6	10.0	0	58 (6.5)	
			0.3[c]	❑ ala	5	a.3	0	66 (5.3)	
				female	15	25.0	0	65 (4.3)	
			0.4	male	0	0	0	---	
				female	0	0	0	---	

[a] This excludes 1 fish which was not measured for length.
[b] This excludes 6 fish which were not sexed.
[c] This excludes 2 fish which were not sexed.

Thompson (1987) suggests a sample size of 510 ageable fish to estimate age composition given 3 or more age classes with multinomial distribution, an alpha level of 0.05, and a minimum detectable difference of 0.05 between the sample estimate and the true population age proportion. Sample sizes this large are unlikely to be collected within the Yakima River. Based on Thompson's recommendations, a minimum of at least 150 ageable fish per stratum (e.g. substock baseline sample or fishery sample) should be collected for preliminary age composition estimation. This will result in at least 95 percent probability that each

estimated age proportion will be within a minimum detectable difference of 0.09 of the true proportion given three or more age classes. In order to meet the minimum 150 ageable fish goal, at least 200 fish should actually be sampled to compensate for fish with unusable scales due to regeneration, missing samples, and scales mounted upside down. Six scales per fish should be collected in order to reduce regeneration rates (Knudsen 1990).

Yakima Indian Nation (YIN) personnel collected spawning ground scale samples in addition to the fish which were GSI sampled. However, an unknown number of these fish were scale sampled by both WDF and YIN personnel. Increasing the sample size by including YIN samples will improve the precision of age estimates and also extend the temporal representation of most groups. In the future it is recommended that some technique be used to identify carcasses or live fish which have been scale sampled by either WDF or YIN personnel, e.g. cutting caudal fins of sampled fish, so that no duplication of effort occurs.

Sex Ratios

Male to female ratios in Naches system samples were nearly 1:1 (1989 weighted male mean 53 percent; range of 44 to 59 percent; 1990 weighted male mean 41 percent, range of 33 to 60 percent). The other Yakima River spring chinook groups were more heavily weighted toward females (1989 and 1990 weighted male mean 35 percent; male range of 8 to 46 percent).

Marion Drain fall chinook samples were primarily male (71 and 65 percent male in 1989 and 1990, respectively) due primarily to the large proportion of jacks. Conversely, Benton City fall chinook were 33 and 43 percent male in 1989 and 1990, respectively.

Mean Aae of Female Reproduction

Healy and Heard (1984) found that mean age of female reproduction was positively correlated with fecundity at a standardized length of 740 mm in chinook. "Mean age of female reproduction" is defined here as the mean age of recovered female carcasses within a group, with "age" being defined as the total number of completed years of life, i.e. an age 1.3 fish has an "age" of 4 years.

Mean age of female reproduction for Naches system spring chinook ranged from 3.3 to 3.9 years, while other Yakima River spring chinook groups ranged from 3.0 to 3.1 years (Table 16). Female Marion Drain fall chinook samples had a mean reproductive age of 2.0 and 2.7 years in 1989 and 1990, respectively, while the Benton City 1990 sample had a mean of 3.0 years (Table 17). The 1989 Benton City sample is not considered since it contained only 6 fish. Based on Healy and Heard's results, this data would suggest

that Naches system spring chinook are more fecund at a standard length than other spring chinook groups within the Yakima system and that spring chinook should be more fecund than Marion Drain fall chinook at a given length. In addition, Benton City fall chinook fecundity may be comparable to mainstem and upper Yakima spring chinook, based on their similar mean age of female reproduction.

Mean age of female reproduction also influences effective population size. The mean age of female reproduction or generation length in Pacific salmon is directly related to the number of effective spawners in a population (Waples et al. 1990), that is, the number of effective breeders times the average age of female reproduction equals the effective population size. Thus, mean age of female reproduction is a variable affecting a significant facet of reproductive success (fecundity) and population genetics (effective population size) and should be monitored over time to determine pre-supplementation values and year to year variation within the identified substocks. Post-supplementation monitoring of age and sex composition should continue in order to document any change in mean female age of reproduction that may occur.

Spawning ground recovery rates of chinook, chum (0. *keta*), coho (0. kisutch) and sockeye (0. *nerka*) salmon carcasses have been shown to be biased in many, though not all, studies (Peterson 1954, Clutter and Whitesel 1956, Eames et al. 1983, Sykes and Botsford 1986). Typically, larger fish are recovered at higher rates than smaller fish and females at higher rates than males due to larger fish being bigger "targets", female behavior patterns that make them less likely to be washed downstream after dying, and predators and currents removing smaller fish from recovery areas at higher rates. This can result in large females being recovered at 4 times the rate of small males (Clutter and Whitesel 1956). Stream morphology and water clarity will also influence recovery rates. The 1989 data presented above exhibit no trend in the male to female ratios across all the groups that would indicate a consistent bias toward higher recovery rates for females in all streams. If there is a male/female recovery rate bias, it is stream specific and, without knowing the true age and length distributions by sex for individual streams, the bias cannot be estimated. We do have estimates of the percentage of wild spring chinook jacks passing upstream at both Prosser and Roza in 1989 (6 percent at both facilities) based on fish size recorded on video tapes (Mike Cohn, YIN, pers. comm., 1990). However, no test of the accuracy of the video tape age estimates has been made and the Prosser right bank ladder tends to pass fish of smaller size than the other two ladders (B. Watson, YIN, pers. comm.). Six percent of the 414 aged spring chinook represents 25 jacks, however only 6 jacks were actually recovered on the spawning grounds. There is a significant difference between the observed and expected jack recoveries in 1989 (X^2= 10.8 with Yates correction, df=1, P=0.001), indicating spring chinook jacks

may be under represented in the spawning ground samples, provided the estimate of 6 percent jacks at Prosser and Roza is accurate.

Scale Pattern Analysis of 1989 Natural Spring Chinook Groups

Two-way ANOVA of scale measurements was used to estimate group (American, Naches, and Little Naches), age (1.2 and 1.3), and interaction effects (Table 18). Groups having less than 10 fish within each group/age cell were not included. Significant group effects were found for variables T1, T4, T8, and T9 ($P \leq 0.01$) indicating there may be some potential for group discrimination based on scale patterns, although statistical significance alone does not imply high classification accuracy. No variable had a significant age effect ($P \geq 0.08$) and only variable T3 had a significant group/age interaction effect ($P < 0.01$). Since age was not a significant factor, ages were pooled within the spring chinook groups in the analyses below. Scale variable means for each group are given in Table 19.

Table 18. Results of two-way ANOVA of group (American, Little Naches, and Naches rivers), age (1.2 and 1.3), and group/age interaction source effects for 12 scale variables (see Table 15 for definitions of scale variables).

Scale variable	Probability values for source effects		
	Group	Age	Interaction
Focus	0.59	0.66	0.48
T1	**<0.01**	0.31	0.81
T2	0.07	0.08	0.46
T3	0.32	0.33	co.01
T4	0.01	0.83	0.18
T5	0.09	0.17	0.92
T6	0.24	0.51	0.22
T7	0.16	0.37	0.26
T8	**<0.01**	0.91	0.63
T9	0.01	0.49	0.82
T10	0.39	0.67	0.92
T11	0.84	0.68	0.99

Table 19. Spring chinook salmon scale variable means and standard deviations in microns by group (pooled over ages 1.2 and 1.3). The Leavenworth Hatchery group is included for comparison purposes. Scale variables are defined in Table 15.

	Mean in microns (sd) by group							
Scale variable	American	Bumping	Naches	L Naches	Cle Elum	Easton	Below Rosa	Leavenworth H
Focus	79 (11)	85 (10)	77 (11)	79 (11)	79 (11)	79 (11)	80 (8)	80 (11)
T1	93 (16)	88 (12)	83 (12)	83 (12)	88 (13)	83 (15)	93 (14)	91 (13)
T2	68 (14)	63 (12)	64 (10)	64 (10)	68 (11)	62 (10)	64 (8)	71 (10)
T3	60 (12)	59 (9)	58 (10)	61 (11)	61 (10)	55 (9)	56 (15)	70 (12)
T4	59 (10)	53 (4)	53 (9)	59 (10)	54 (10)	52 (11)	57 (10)	72 (14)
T5	62 (13)	69 (11)	S6 (10)	60 (14)	58 (15)	59 (15)	65 (23)	72 (14)
T6	70 (17)	71 (10)	65 (14)	70 (19)	71 (19)	72 (20)	73 (14)	64 (14)
T7	81 (25)	87 (18)	74 (16)	83 (17)	76 (16)	82 (19)	79 (24)	66 (13)
T8	101 (29)	101 (28)	04 (18)	86 (23)	93 (28)	90 (23)	87 (18)	78 (22)
T9	117 (33)	118 (35)	100 (26)	107 (19)	109 (32)	109 (26)	114 (32)	101 (33)
T10	127 (25)	116 (24)	123 (28)	129 (26)	118 (27)	121 (23)	121 (31)	132 (29)
T11	126 (31)	134 (25)	128 (24)	130 (24)	132 (21)	120 (21)	123 (24)	137 (23)

A LDF analysis of the 7 natural spring chinook groups resulted in jackknifed classification accuracies which were generally very low (≤27 percent correctly identified for any group; Table 20), indicating that accurate composition estimates for each of these groups are not possible. Jackknifing is one method of reducing bias in the estimated misclassification rates and is most effective when the sample sizes for standards are relatively small, as they are in this particular analysis.

Table 20. Jackknifed classification matrix for the 7 natural groups of spring chinook. Classification results along the underlined diagonal are the percentage of fish correctly classified into each group.

	Percentage of fish classified into each group							Sample
Group	Amer	Bump	L Nach	Nach	C Elum	Easton	B Rosa	size
American	17	9	12	19	5	7	31	58
Bumping	9	27	9	0	9	18	27	11
L. Naches	13	13	26	23	6	10	10	31
Naches	9	5	20	18	16	18	13	55
Cle Elum	18	18	10	12	22	6	14	50
Easton	4	19	11	13	17	26	9	53
Below Roza	33	22	0	0	11	2 2	11	9

Mean unweighted classification accuracy = 21 percent

Table 21. Euclidean distance between pairs of 1989 spring chinook groups in seven-dimension canonical variate space. Distances are dimensioned in pooledwithin-group standard deviations.

Group	Distance between paired groups							
	American	Bumping	L Naches	Naches	Cle Elum	Easton	Below Rosa	Leavenworth H
American R.	0.00							
Bumping R.	1.46	.00						
L Naches R.	1.15	1.47	.00					
Naches R.	1.02	1.60	.78	.00				
Cle Elum R.	0.85	1.39	1.02	.79	.00			
Easton	1.13	1.32	.91	.78	1.02	.00		
Below Roza	0.97	1.37	1.21	1.23	1.21	1.07	.00	
Leavenworth H	2.20	2.69	2.11	2.30	2.31	2.75	2.18	.00

Between-group comparisons of naturally rearing 1989 return Yakima River and 1988 return Leavenworth Hatchery (Wenatchee River) spring chinook scale patterns were made using plots of the first and second CV. Leavenworth Hatchery spring chinook were chosen because they were felt to be a reasonable representation of how the scale patterns of supplemented spring chinook in the Yakima River might appear. The first and second canonical variables explained 91 percent of the total variation. Plots of the first two canonical variables are shown in Fig.7. Between-group distances in canonical variate space are given in Table 21. Between-group distances within the natural groups were generally small (mean between-group distance = 1.1) and parallel the low classification accuracies of natural groups seen in Table 20. Distances between the Leavenworth Hatchery and natural groups were much greater (mean between-group distance = 2.4). Since it is not possible to discriminate accurately between the naturally rearing groups based on scale patterns, these groups should be combined into one large natural group in future analyses.

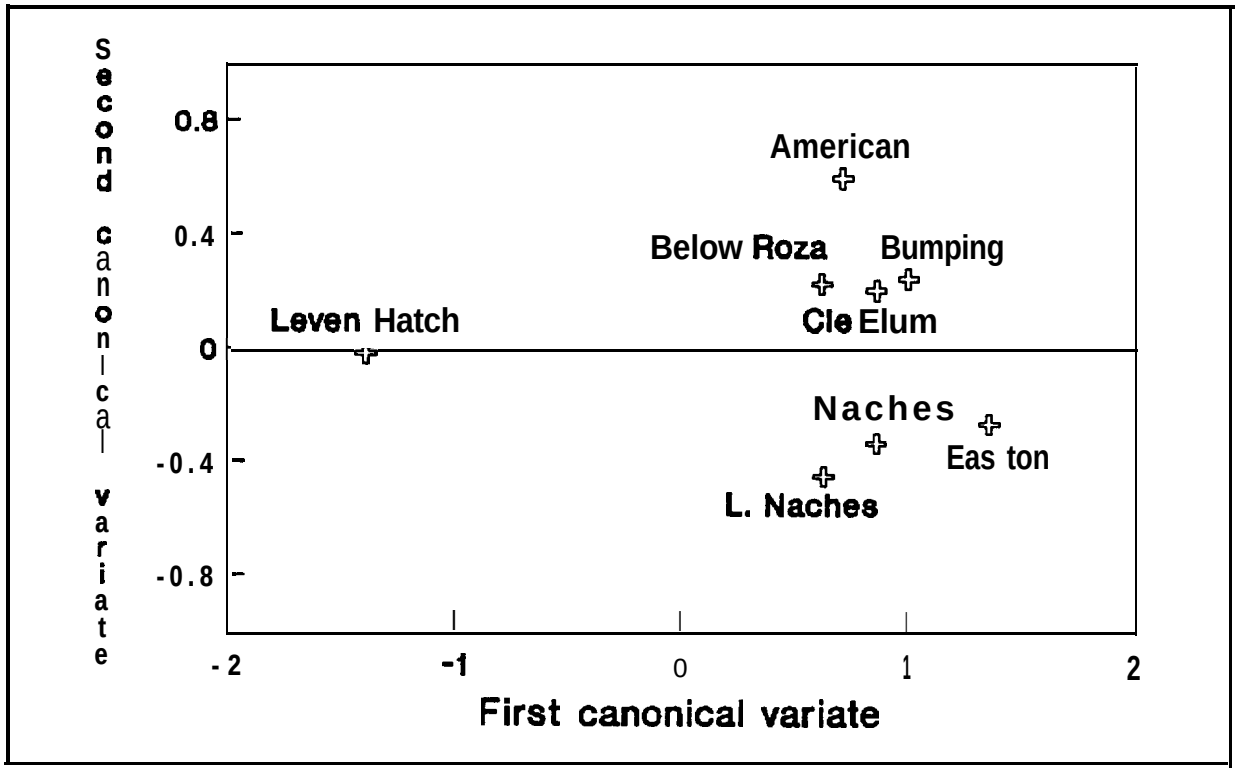

Fig.7. Group centroids for 1989 Yakima River natural and 1988 return Levenworth Hatchery spring chinook based on canonical variates analysis of scale patterns.

The similarity in scale patterns between natural spring chinook groups is probably due to juveniles from different natal streams experiencing considerable overlap in rearing habitat resulting in similar patterns of growth. It is believed that about 30 percent of American River fry begin to distribute themselves downstream out of the American River soon after emergence (D. Fast, YIN, pers. comm., 1990). This general trend of juvenile spring chinook moving downstream early in their life is true throughout the Naches system where apparently each year 50 to 80 percent of the total Naches outmigration passes Wapatox in late fall and early winter (J. Hubbel, YIN, pers. comm., 1990). Significant fall and winter downstream movement of juvenile spring chinook has been observed at Roza, as well. As this mixture of fish from different natal streams moves downstream, they experience similar rearing conditions. Thus, individuals from different natal streams grow at comparable rates developing scale patterns which are more similar than would be expected had they reared their entire juvenile freshwater period isolated within their respective natal streams.

Natural and Hatchery Spring Chinook Group Identification Analyses

A two-way LDF analysis of 1988 return Leavenworth Hatchery and pooled Yakima River natural spring chinook groups was made. The percentage of fish correctly classified into the Leavenworth Hatchery and natural group was 83 and 90 percent, respectively. Simulation analysis showed that over a wide range of mixing proportions estimates of hatchery and natural group contribution were unbiased with overall mean known composition and estimated composition equalling 50.4 percent natural and 49.6 percent hatchery, respectively. The standard deviation of the errors was 5 percent or approximately 95 percent of the estimates were within 10 percent of the true value.

LDF analysis of scale patterns of other Columbia River hatchery and natural spring chinook have resulted in classification accuracies ranging from 81 to 95 percent (Knudsen and Sneva 1989, Sneva and Knudsen 1989, Fryer and Schwartzburg 1990), similar to the Yakima natural/Leavenworth Hatchery results above. Thus, it is highly probable that scale patterns of hatchery spring chinook reared as yearlings will differ significantly from scale patterns of naturally rearing spring chinook and scale pattern analysis will give accurate estimates of the proportion of natural and hatchery fish in a Yakima River mixed-group sample once supplementation begins.

Elemental Analysis of Scales for Background Levels of Trace Elements.

Recent work in elementally marking mineralized tissues, particularly scales and otoliths, has shown that the technique has the potential for use as a mass marking technique (Behrens Yamada and Mulligan 1990, Coutant 1990). Naturally occurring differences in trace element concentrations in scales have been used to identify populations of wild sockeye salmon, as well (Lapi and Mulligan 1981). Coho salmon scales have been marked with a stable strontium enriched diet and the mark recovered from adult returns 18 months later (Behrens Yamada and Mulligan 1982). Recent work at WDF on newly emergent chum and sockeye fry has shown that otoliths and backbones can be successfully marked with a stable isotope of strontium by immersing fish for 24 hours in 125 to 9,000 mg/L strontium chloride solutions (Steve Schroder, WDF, pers. comm., 1990). An inductively coupled plasma (ICP) mass spectrometer was used to detect the elevated concentrations of strontium in otoliths and vertebrae in chum and sockeye fry 6 weeks after marking. A recent innovation in laser microprobe analysis has made it possible to analyze very small portions of scales or other hard parts and identify concentrations of trace elements that would otherwise be undetectable due to dilution by the surrounding tissues (Coutant, Oakridge National Lab, pers. comm., 1991). WDF and Oakridge National Lab, in cooperation with CRITFC, are currently writing a

joint BPA proposal to fund research employing and further developing this new microanalytical technology to identify natural substocks of spring chinook throughout the Columbia River drainage and mass mark experimental and control release groups.

Elements that occur in high concentrations naturally may be useful in identifying natural substocks and separating early from late migrating spring chinook. The baseline data for the naturally rearing groups will also be used to assess which elements will likely discriminate hatchery from naturally rearing spring chinook as both juveniles and adults. Elements which have low concentrations in natural substocks, low variance within groups, and can easily be incorporated into the scales of hatchery fish will be preferred elements for marking hatchery releases.

Representative scale samples (n=248) for elemental analysis were collected from Yakima River spring chinook groups in order to determine the background levels of trace elements in scales (Table 22). Samples of scales (12 per fish) were removed from the preferred area and placed into coin envelopes for storage. Sample location, length and sex were recorded on each envelope. Elemental analysis of trace elements in adult Yakima River spring chinook scales will be done as time and funds become available. Part of the BPA proposal with Oakridge National Laboratory will include trace element analysis of natural Yakima spring chinook scales.

Table 22. Scale sample collections from 1989 returning Yakima River spring chinook for determination of naturally occurring concentrations of trace elements.

Group	Number of fish scale sampled
American River	56
Bumping River	26
Naches River	45
Little Naches River	37
Easton	37
Cle Elum River	37
Below Roza	10
Total sample size	248

SUMMARY RECOMMENDATIONS

♦ A sample size of 200 fish per stratum (i.e. substock baseline sample or fishery sample) is recommended as a target sample size for preliminary age composition estimates in order to insure relatively precise age composition estimates for these strata. Samples of this size probably cannot be collected at this time from

spawning ground recoveries even with tremendous effort but may be possible at some future time from spawning grounds and fisheries.

♦ Continue to collect age/length/sex data from sub-stocks in order to determine pre-supplementation mean age of reproduction, age composition, length-at-age, and male/female ratios.

♦ Construct a weir(s) or utilize an existing adult monitoring site(s) which allows all adult fish passing upstream to be counted and a known percentage jaw tagged. Compare the sex composition, age composition, and length-at-age of jaw tagged recoveries made using standard spawning ground survey techniques to the original tagged sample released at the weir in order to determine whether spawning ground recovery data is biased and, if so, how great the bias is.

♦ Follow up the mean age of female reproduction analysis by collecting fecundity information from in-river chinook fisheries. At a minimum, this will allow refinement of our current estimates of the length-fecundity relationship through increased sample size. Methods of separating in-river caught spring chinook females into Naches and upper river groups based on sub-stock differences in female length-at-age should also be explored. Possible differences in fecundity between Naches system and upper Yakima sub-stocks may then be estimated. By collecting samples from in-river fisheries the need to remove pre-spawning fish from the spawning grounds is eliminated. GSI analysis will be useful as a method of testing the accuracy of the age/length sub-stock identification method. The proportion of Naches system and upper Yakima groups present in a mixture can be accurately estimated using GSI (see Chinook GSI section), although individual fish cannot be accurately identified with this technique. If GSI analysis confirms the separation based on female age and length, then we can be more confident in our results. However, if GSI analysis identifies significant proportions of upper Yakima fish within the sample identified from length-at-age analysis as being of Naches origin, we can assume there are problems with the age/length analysis.

♦ Develop a method of aging adult spring chinook using image analysis of video tapes of fish taken as they pass upstream at adult monitoring facilities. Length (POHP) was shown to accurately predict age in Yakima spring chinook and a method of measuring POHP length from video images of adults as they pass upstream could be developed using image processing technology presently used by WDF. This method could be used to more accurately identify the number of jacks passing upstream, provide broad based across **substock** estimates of age composition, and identify temporal trends in migration by age/size class.

♦ It is recommended that some technique be used to identify carcasses or live fish which have been scale sampled by either WDF or YIN personnel, e.g. cutting **caudal** fins of **sampled** fish, so that no duplication of effort occurs.

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YAKIMA STEELHEAD AND RAINBOW TROUT AGE, LENGTH AND SCALE PATTERN ANALYSES.

INTRODUCTION

The following report describes age and growth analyses of Yakima River steelhead and rainbow trout related to the prefacility phase of the Yakima/Klickitat Production Project (YKPP). The central hypothesis of the YKPP is that new artificial production in the Yakima River can be used to increase harvest and to enhance natural production without adversely affecting genetic resources (EDWG 1990). The pre-implementation process requires the characterization of existing substocks in terms of genetics, life history traits, growth, and age and also addresses the need to explore methods for identifying existing substocks. Species interaction studies have identified a need to separate juvenile steelhead from resident rainbow trout, as well.

The purpose of this report is to describe the age composition of juvenile steelhead trout collected in the spring of 1989 and 1990 and adult steelhead collected for broodstock purposes in 1989/90. Scale pattern analysis is explored as a method to discriminate substocks of steelhead‘trout and to discriminate steelhead from resident rainbow trout. Length is evaluated as a method for ageing smolts passing Prosser. Estimates are made of the age composition of resident rainbow trout collected in 1990 and the amount of body length shrinkage occurring during freezer storage, In addition, the size of scale samples collected for elemental analysis are reported.

METHODS

Sample Collection and Ageing

Steelhead smolt and rainbow trout scale samples and fork lengths were collected by Washington Department of Fisheries (WDF) personnel from frozen samples collected in conjunction with genetic stock identification (GSI) analyses, Rainbow trout scale samples and fork lengths were also collected by Washington Department of Wildlife (WDW) personnel. Twelve or more scales per fish were generally collected from the INPFC preferred body area. Acetate impressions of the mounted scales were made (Clutter and Whitesel 1956) and used in ageing and scale pattern measurements.

All fish were aged visually using acetate impressions of scales under a micro-fiche reader at 24 and 48 X. A subsample of adult and juvenile scale samples were aged jointly by Bob Leland, WDW, and Curtis Knudsen, WDF, and methodologies were informally compared to insure that ageing techniques and criteria were consistent between analysts. The European form of age designation is used for adult

ages in this report (Koo 1962). For example, age 1.3 designates that one winter was spent in freshwater (the numeral to the left of the decimal point) and 3 winters were spent in the ocean (the numeral to the right of the decimal point). Juvenile fish which have completed one year are designated age 1+, two years age 2+, etc

Steelhead smolts were collected at Prosser, Wapatox, and Roza diversion dams and Satus, Logy, and Dry creeks for GSI and scale analysis purposes by Yakima Indian Nation (YIN) personnel. Adult steelhead scale samples (n=52 fish) were collected from broodstock taken between 10/18/89 and 1/23/90 at Prosser Dam. Fork length, date of capture, and the sex of each fish were recorded.

SCALE PATTERN MEASUREMENTS

Scale pattern measurements to the 34th circulus were made using the same equipment and techniques employed in the spring chinook scale pattern analyses (see Chinook Substock Identification section). The twelve variables measured are given in Table 23. In some cases, small fish were not large enough to have 34 complete circuli on their scales. In such cases the last one or two intercirculus measurements were dropped so that only complete triplets were used. These fish would then have less than eleven triplet variables describing their scale patterns.

Table 23. Scale character variables used in scale pattern analysis for group discrimination. All variables are distance measurements in microns.

Variable name	Definition
Focus	The distance from the center of the focus to the outer edge of the first circulus.
T1	The first intercirculus triplet beginning at the outer edge of the first circulus and extending out to the outer edge of the fourth circulus.
T2, T3, ...,T11	The distance from the outer edge of circulus ((3*i)-2) to the outer edge of circulus ((3*i)+1), where i equals 2, 3,..., 11 and i represents the triplet of interest.

Linear Discriminant Function (LDF), Canonical Variates (CV), and Simulation Analyses

The LDF and CV analyses were performed using the same software and statistical techniques used in the spring chinook analyses. Simulation analyses were performed on steelhead scale pattern data to assess the accuracy of group identification models over a wide range of possible mixing proportions (see Chinook Substock Identification section for details).

Each simulation involved creating standards and a mixture, calculating a classification rule, classifying the mixture sample, correcting the classification results, and if necessary constraining the estimates. This process was repeated from 234 to 400 times (depending on the analysis) and the error in the estimated proportions for each mixture were saved. Error is defined as the difference between the known and estimated proportion of each group in a mixture. The mean of the errors is a measure of bias and the standard deviation of the errors is a measure of precision of the model.

Scale Collections for Elemental Analysis

Approximately 1 mg of nonregenerated scale material was scrapped from the preferred body area and immersed in deionized water for approximately 24 hours. Scales were removed from the water and non-regenerated scales sorted out. The remaining scales were then allowed to air dry for approximately 24 hours. The scales were then placed into individually capped and labeled 3 ml beaker cups for storage and eventual analysis.

Steelhead smolts from Wapatox (n=50 with 5 replicate samples), Roza (n=50 with 9 replicate samples), and Dry Creek (n=50 with 9 replicate samples) were scale sampled. No hatchery reared smolts were collected in 1989. There are 1990 hatchery smolt samples available, although scales for elemental analysis have not been collected from these samples at this time. Least-squares linear regression analysis of the number of scales per mg of scale material versus fish length was used to estimate the number of scales needed from a fish of a given length to makeup a 0.5 mg scale sample. Fish from the 1989 Wapatox sample ranging in length from 142 to 202 mm were scale sampled for this analysis. Scrape samples from the preferred body area were made and between 24 and 193 non-regenerated scales were collected. Weights of dry scale material were measured to the nearest mg.

RESULTS AND DISCUSSION

Juvenile Steelhead Aae Distributions

Age distributions for the 1989 Roza, Wapatox, Dry Creek, and Prosser smolt samples and 1990 Wapatox, Logy, and **Satus** smolt samples are given in Table 24. Prosser samples were divided into four arbitrary temporal groups for comparison of age distribution changes in the outmigration over time. The 1989 Roza and Wapatox and 1990 Wapatox age distributions were not significantly different (X^2=2.62; df=4; P=0.62) indicating that upper river populations in 1989 had similar age distributions and between year differences were not significant in the Wapatox samples. The 1989 Wapatox and Roza age distributions were then combined and compared to the 1989 Dry Creek age distribution. This comparison showed a significant difference between 1989 upper and lower Yakima River age distributions (X^2=87.0; df=2; P<0.001) with the lower river. Dry Creek sample having a much higher proportion of age 1+ fish. The 1990 **Satus** and Logy creek ages were not significantly different (X^2 with Yates **correction**=0.1; df=1; P=0.78) and so were combined and compared to the 1990 Wapatox ages. This comparison showed that there were significant differences between the lower river (**Satus** and Logy creeks) and upper river (Wapatox) ages (X^2=87.1; df=2; P<0.001), although the difference was not as great as in the 1989 samples.

Table 24. Age distributions for Yakima River steelhead smolts captured in 1989 and 1990.

Year	Group	Freshwater age (percent) 1+	2+	3+	4+	Sample size
1980						
	Dry Creek	72	27	1	0	a9
	Roza	11	71	18	0	38
	Wapatox	11	78	13	0	145
	Prosser					
	5/3-511	47	44	9	0	88
	5/14-5/18	61	35	4	0	49
	5/22-5/31	54	42	4	0	91
	6/2-6/13	41	52	5	2	42
	Pooled over all dates	50	44[a]	6	0[b]	272
1990						
	Logy Creek	26	74	0	0	73
	Satus Creek	29	69	2	0	96
	Wapatox	17	73	10	0	94

[a] Includes three fish with unknown time of capture.
[b] Value is less than 0.5 percent.

There was no significant difference in age distributions between the four temporal samples collected at Prosser (X^2=6.2; df=6; P=0.41), indicating no significant trends in time of outmigration

past Prosser for the three principle age classes of steelhead smolts in 1989. There was significant genetic heterogeneity found in the Prosser samples (see Steelhead Substock Identification section), indicating that more than one population was migrating past Prosser within each temporal period. Significant temporal changes in allele frequencies were also noted indicating that either the proportional representation of populations was changing over time or different populations were represented in each temporal stratum. The relatively stable age composition of the Prosser samples over time would indicate that the most abundant groups moving past Prosser have very similar age compositions.

The 1990 Logy and Satus creek samples have a significantly greater proportion of age 2+'s and a lower proportion of age 1+'s than the 1989 Dry Creek sample ($X^2=17.3$; df=2; $P<0.001$). In order to avoid bias in this X^2 test, the few age 3+ fish in each group were pooled with the age 2+'s. Since no 1990 Dry Creek or 1989 Satus or Logy creek samples were taken, it is not possible to compare between years within a group and determine whether the difference in Satus system age distributions is due to between year variation or between population variation. However, it is known that Dry Creek experiences periods of low flows when portions of the creek are completely dewatered creating isolated pools leading to increased mortality and poor growth, while Logy Creek does not generally follow this trend (J. Hubbel, YIN, pers. comm., 1990). Natural selection should favor fish that minimize the number of years exposed to these conditions. Those fish spending 2 or 3 years in Dry Creek should experience higher rates of mortality. Thus, there is some question as to how representative Dry Creek smolt ages are of the Satus system in general due to possible differences in the two freshwater environments and resulting differences in growth and survival.

Histograms describing the length frequency distributions of 1989 Wapatox, Roza, Dry Creek, and Prosser samples are shown in Figs.8-11. There is considerable overlap in the lengths of all three age classes in the Prosser sample (Fig.11) and it appears that estimates of age from length are not likely to be accurate. The accuracy of estimating age from smolt length data at Prosser using linear discriminant function (LDF) analysis was tested using computer simulations (see Ageing Steelhead Via Length Distributions section below) and was found to be unreliable.

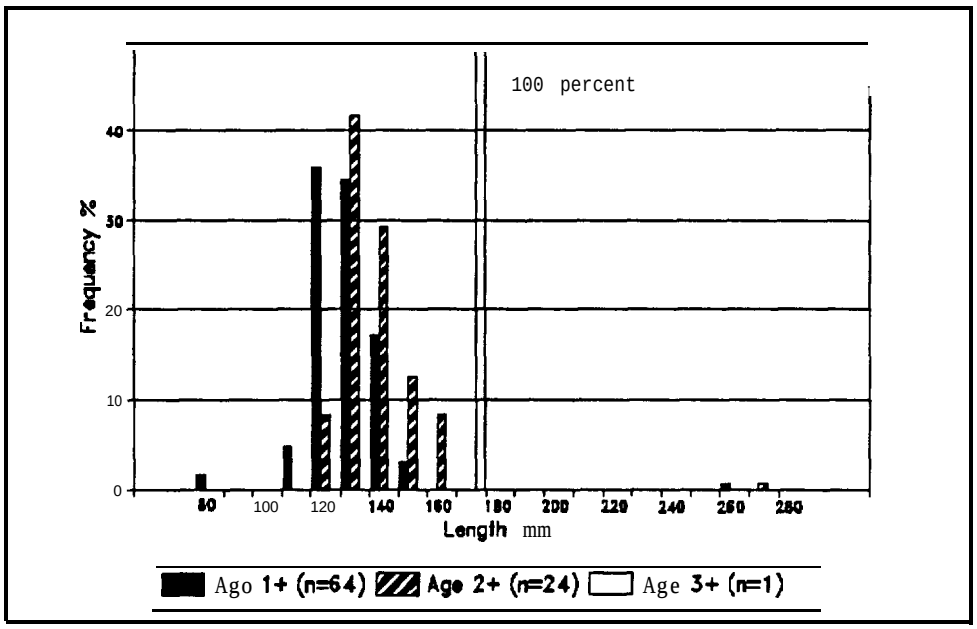

Fig.8. **Dry** Creek 1989 length frequency distributions and sample sizes (n) for steelhead ages **1+, 2+,** and **3+.**

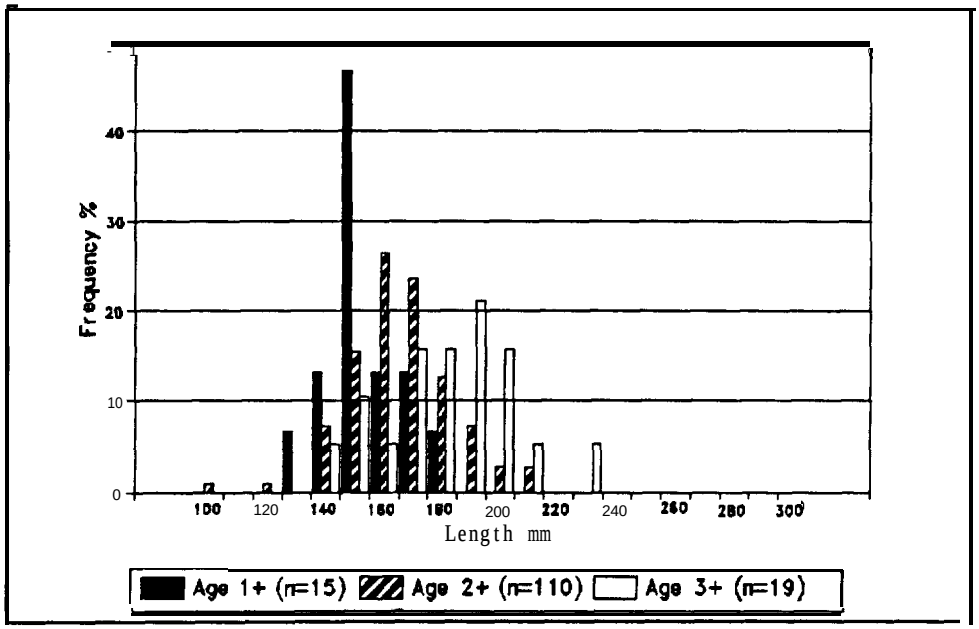

Fig.9. Wapatox 1989 length frequency distributions and sample sizes (n) for steelhead ages **1+, 2+,** and **3+.**

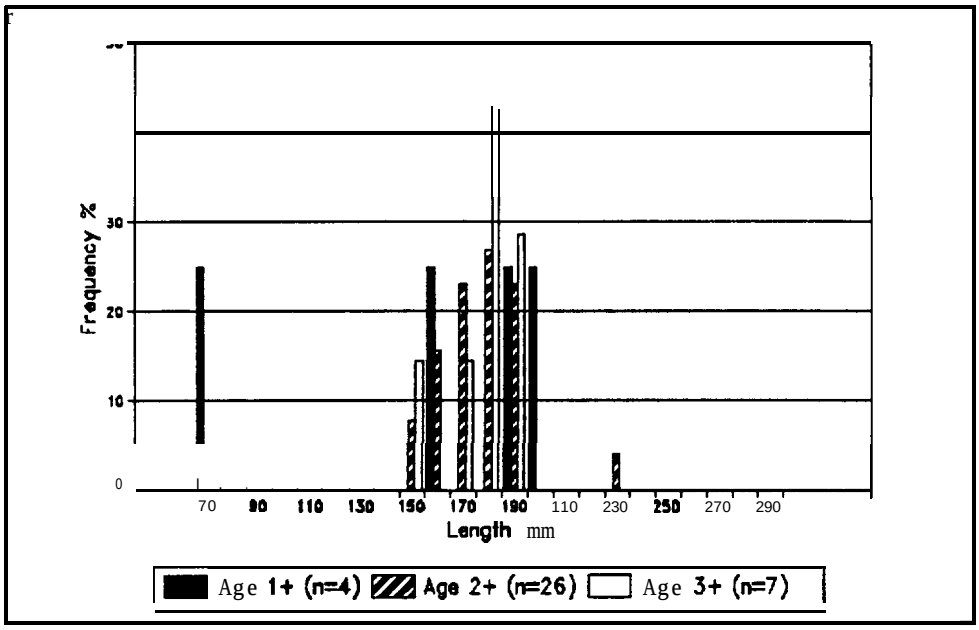

Fig.10. Roza 1989 length frequency distributions and sample sizes (n) for steelhead ages **1+. 2+** and **3+.**

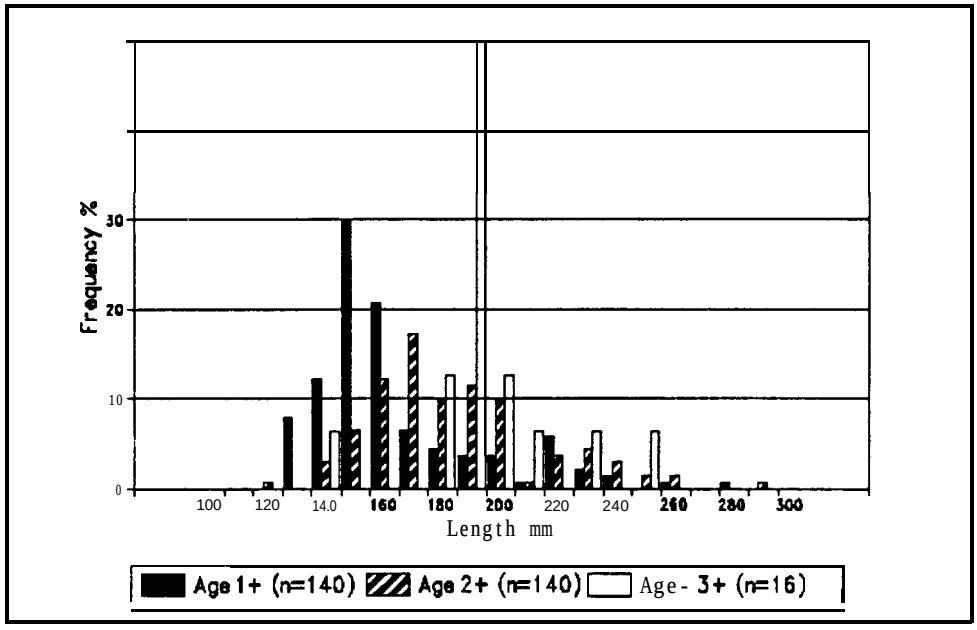

Fig.11. Prosser 1989 length frequency distributions and sample sizes for steelhead age **1+. 2+** and **3+.**

Adult Steelhead Broodstock Acres

Adult steelhead scale samples (n=52 fish) were collected from broodstock taken between 10/18/89 and 1/23/90 at Prosser Dam. Fork length, date of capture, and the sex of each fish were recorded. Twelve scales per fish were collected from the preferred INPFC body area (Major et al. 1972) and placed into a coin envelope for later mounting onto gummed scale cards. Acetate impressions of the mounted scales were made (Clutter and Whitesel 1956) and used in ageing and scale pattern measurements.

Adult age composition for steelhead broodstock collected at Prosser Dam is given in Table 25. Freshwater age 2+ adults made up the greatest portion of the broodstock representing 81 percent of the sample, while age 1+ and 3+ fish each made up 8 percent. Ocean age .1, .2, and .3 fish made up 52, 42, and 6 percent of the sample, respectively. Ocean ages include regenerated scale samples for which no freshwater age could be determined but ocean age could be determined. Repeat spawners were estimated to make up 12 percent of the sample (n=6).

Table 25. Adult age composition of broodstock collected at Prosser Dam between 10/18/89 and 1/23/90. Regenerated scales had unknown freshwater ages, but ocean ages could be determined.

Number and percentage of usable fish in each age category									Total aged	Resenerated		
	1.1	1.2	2.1	2.2	2.3	3.1	3.2	4.1		.1	.2	.3
n												
%	21	36	21 44	33 16	24	24	24	21	48	24	11	12

The 1989 adult steelhead broodstock most closely resemble Satus Creek juvenile steelhead electrophoretically (see Steelhead Substock Identification section). However, the freshwater age composition of Satus system juveniles sampled in both 1989 and 1990 was much more heavily weighted toward freshwater age 1+ fish than the returning adult broodstock collected in 1989/90. There are at least three possible reasons the sample of adult returns does not have a similar age composition as Satus system juveniles given that it genetically most closely resembles Satus system fish. First, the adult return sample may represent a tributary or tributaries within the Satus system which produce much higher proportions of age 2+ smolts than Satus and Logy creeks. Second, the adult returns may be dominated by two particularly strong brood years in which the proportion of age 2+ outmigrants was very high (large interannual variation in the proportion of age 1+ and 2+ smolts leaving the system). Finally, the adult return sample may be made up of age 2+ smolts from throughout the entire Satus system which survived after outmigration at a much higher rate than smaller age 1+ fish.

Aging Steelhead Smolts Via Length Frequency Distributions: Simulation Analysis.

Fork length was evaluated as a tool for estimating the age of steelhead smolts using simulation analysis. The 1989 Prosser smolt length-at-age frequency distributions **(Fig.11)** were used as baseline standards in a 3-way age **1+/2+/3+** model. The proportion of each age class present in 234 simulated mixtures was estimated and the mean error and its standard deviation are given in Table 26. Mean error or bias varied from 0.02 to 0.05 in absolute value and the model's precision, as indicated by the large standard deviations, was very low. Approximate 95 percent confidence intervals around an estimate of 0.50 for either the **1+** or **3+** age classes would extend from nearly 0 to 1 and would actually extend beyond 0 and 1 for age **2+** fish, making these estimates of little practical use.

Table 26. Mean error (known proportion minus the estimated proportion) and standard deviations for each age class over 234 bootstrapped mixtures based on 1989 Prosser steelhead **smolt** age/length frequency distributions (see **Fig.11).**

Aae class	Mean error (sd)
1+	0.017 (0.246)
2+	-0.052 (0.412)
3+	0.034 **(0.222)**

SPA to Identifv Substocks of Steelhead

Typically 12 scale variables are used to describe the first 34 circuli on adult scale samples, however many juvenile samples had fewer than 34 circuli and only the focus and first seven triplet characters could be measured on all fish and these eight variables are used in the statistical analyses below. **Figs.12a-c** shows the mean values for age **1+, 2+,** and **3+ smolts** from Roza, Wapatox and Dry Creek. Mean values for T8 to **T11** in **Figs.12a-c** were calculated using only those fish with complete triplets.

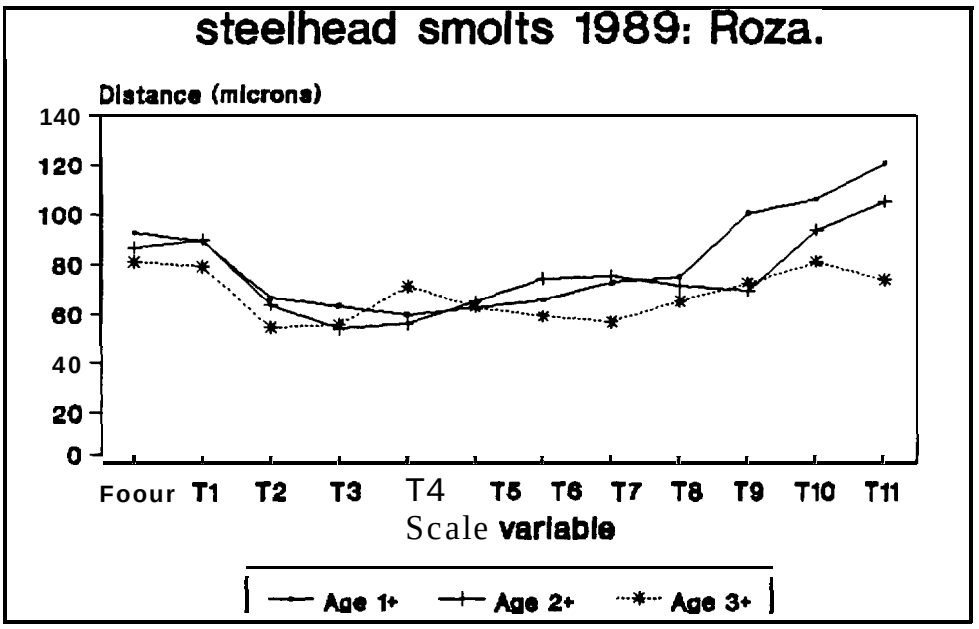

Fig.12a. Scale variable means for 1989 Roza age 1+, 2+, and 3+ steelhead smolts, in microns.

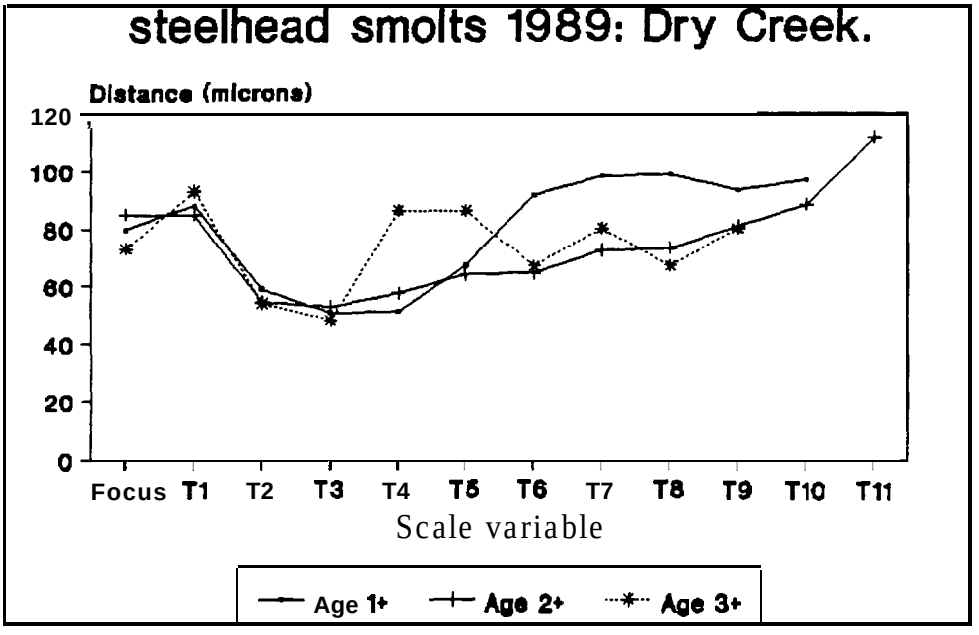

Fig.12b. Scale variable means for 1989 Dry Creek age 1+, 2+, and 3+ steelhead smolts, in microns.

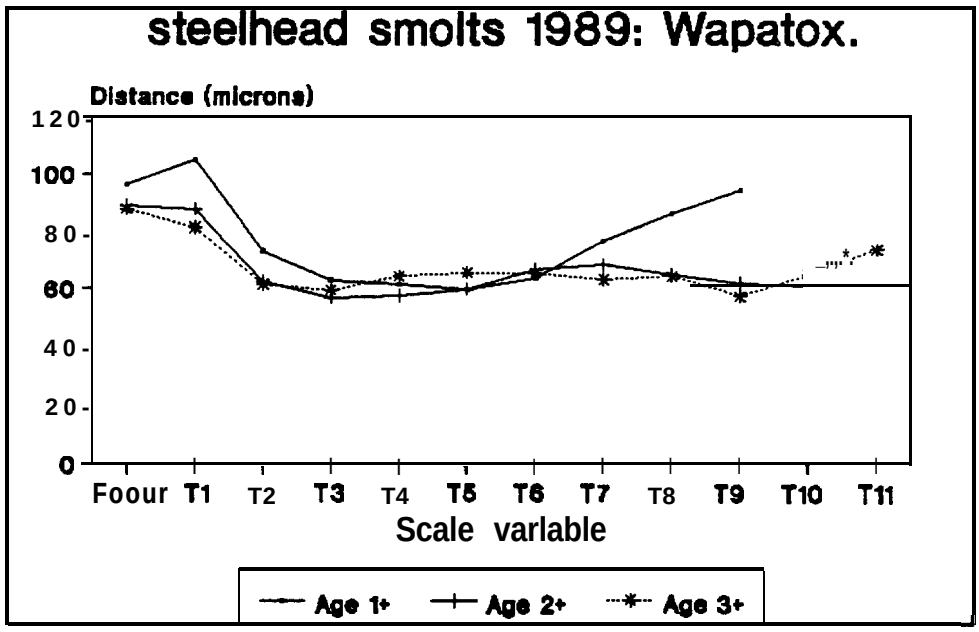

Fig.12c. Scale variable means for 1989 Wapatox age **1+, 2+,** and **3+** steelhead smolts, in microns.

A two-way **ANOVA** of scale characters Focus to T8 was used to estimate group (Wapatox,Dry Creek), age **(1+,2+),** and interaction effects. The **ANOVA** results showed significant group effects in 7 of 8 scale characters and significant age effects in half of the scale characters (Table 27, **P≤0.10).** In addition, 6 characters had significant group/age interactions (PL0.l0). These results indicate that although there are significant differences in scale patterns between groups which might be used to discriminate **them, there** are also significant scale pattern differences between age classes within groups which are as great or greater than between group differences. An additional complication is that between age scale pattern differences are not similar across groups as indicated by the significant group/age interactions. For these reasons each group/age cell must be treated independently. Therefore group/age cells are analyzed independently in the CV and LDF analyses below. The Roza and **age3+** samples were not included in the **ANOVAs** because sample sizes were less than 10 fish per group/cell.

Table 27. Two-way **ANOVA** results for group (Wapatox and Dry Creek) and age **(1+and 2+)** effects for scale characters Focus to T7.

Scale variable	P-value for source effects		
	Group	Acre	Interaction
Focus	0.001	0.334	0.055
T1	0.002	0.020	0.018
T2	0.000	0.005	0.232
T3	0.000	0.010	0.009
T4	0.028	0.862	0.021
T5	0.184	0.944	0.769
T6	0.001	0.151	0.000
T7	0.000	0.002	0.001

Canonical variate analysis of 8 natural and 2 hatchery steelhead groups was performed using the BMDP 7M software (Jennrich and Sampson 1983). The Nile Pond and Nelson Spring age **1+** hatchery steelhead groups are included for comparison purposes. Seven scale characters were forced into the discriminant functions and plots of the first two canonical variates (group centroids) were made.

The first two of seven canonical variates explained 92 percent of the total variation. The group centroids are plotted for the first two canonical variates in **Fig.13.** Between group distances in the seven-dimensional canonical variate space are given in Table 28 and the classification results from a LDF analysis of the same data are given in Table 29. The natural groups' centroids formed one rather loose cluster (mean between-group distance, excluding Dry Creek **1+** and Wapatox age **1+,** -1.5). The Dry Creek age **1+** (mean distance between all other groups= 3.1) and to a lesser extent the Wapatox age **1+** (mean distance between all other groups= 2.5) centroids separated out by themselves. In general for the natural groups, age had a slightly greater effect than group membership on scale patterns since between age (within group) distances **(mean=2.0)** were greater than between group (within age) distances **(mean=1.7).** Centroids for the Nile Pond and Nelson Spring hatchery groups were nearly identical (between-group distance = 0.5) and as was confirmed by an **ANOVA** of the two hatchery's scale characters which resulted in no significant differences between the two hatchery samples **(P>0.09).** Therefore, the two hatchery samples were pooled into a single hatchery group in the next analysis.

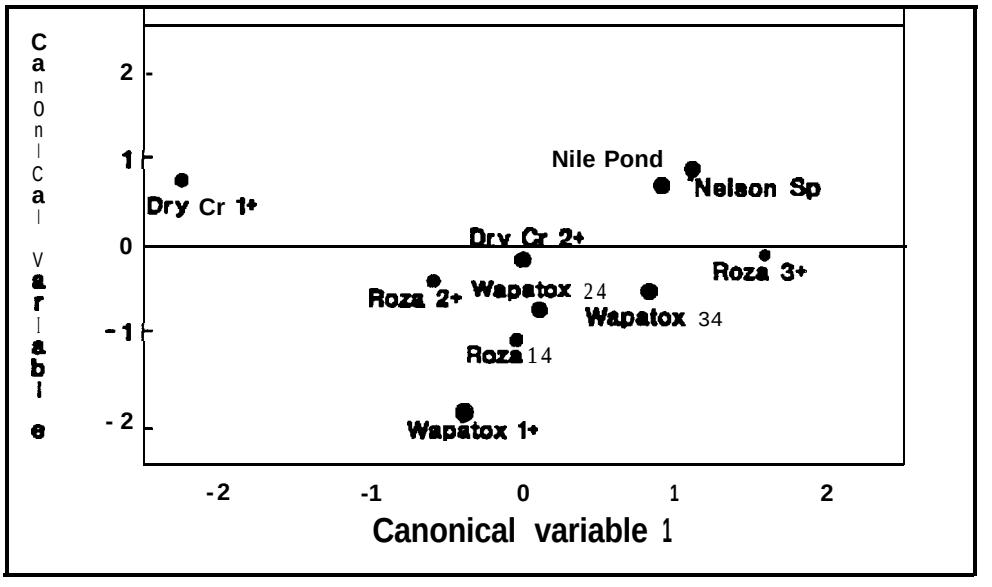

Fig.13. Group centroids for Yakima River 1989 steelhead smolts based on scale variables Focus to T7.

Table 28. Between-group Euclidian distance in seven-dimensional canonical variate space for two hatchery and eight natural steelhead groups.

Group	NelsonSp	NileP	Roza1+	Roza2+	Roza3+	Wapa1+	Wapa2+	Wapa3+	Dry1+	Dry2+
Nelson Spring	.0000									
Nile Pond	.4954	.0000								
Roza 14	2.2643	2.1299	. 0000							
Roza 2+	2.1406	1.9805	1.1609	.0000						
Roza 3+	1.5761	1.6868	2.4159	2.5004	.0000					
Wapatox 1+	3.2986	3.1055	1.2463	1.9397	3.1849	.0000				
Wapatox 2+	1.8852	1.7854	0.7659	0.8379	1.9965	1.7446	.0000			
Wapatox 3+	1.5423	1.4992	1.3637	1.5912	1.3470	2.2986	1.0263	.0000		
Dry Creek 1+	3.4015	3.1936	2.9185	2.0822	4.0812	3.4325	2.7919	3.3854	.0000	
Dry Creek 2+	1.6674	1.5522	1.3038	1.0749	1.9549	2.2748	1.0379	1.4544	2.5396	.0000

Table 29. Classification of results (LDF) for two hatchery and eight natural groups in percent with age classes treated separately. Eight scale variable were forced into the LDF: focus to T7. Classification results along the underlined diagonal are the percentage of fish from each group correctly identified. Sample sizes for each group are given, as well.

Correct group	Classification result in percent										Sample size
	NelsonSp	NileP	Roza1+	Roza2+	Roza3+	Wapa1+	Wapa2+	Wapa3+	Dry1+	Dry2+	
Nelson Spring	37	23	0	1	17	0	4	8	3	7	75
Nile Pond	4 2	18	0	0	6	0	6	9	3	15	33
Roza 1+	0	0	0	0	0	20	20	40	20	0	5
Roza 2+	4	7	7	14	11	18	18	0	14	7	28
Rosa 3+	29	0	0	0	29	0	0	14	0	29	7
Wapatox 1+	0	0	17	0	0	58	8	0	8	8	12
Wapatox 2+	4	9	15	15	4	10	15	10	2	18	82
Wapatox 3+	18	0	12	0	24	6	18	12	0	12	17
Dry Creek 1+	0	5	2	5	2	2	0	0	75	9	55
Dry Creek 2+	10	24	5	5	10	5	24	5	5	10	21

Age and/or group-age interaction effects in the two-way ANOVA above (Table 27) were significant in all but one variable. This contributed to the generally low accuracy with which natural groups could be identified (Table 29). One method of controlling for significant age effects is to divide a sample into its component age classes first and then analyzing the scale measurements of each age class separately in order to estimate the proportion of each substock. In a 3-way LDF analysis of age 1+ Roza, Wapatox and Dry Creek scale patterns, Wapatox and Roza scale patterns were quite similar to each other relative to the Dry Creek sample and misclassified to each other at high rates (Table 30). Based on this result Roza and Wapatox age 1+ samples were combined into one upper Yakima-Naches system standard and a 3-way upper Yakima-Naches/Dry Creek/Hatchery age 1+ model was then constructed and was successful in accurately identifying age 1+ groups. Overall mean classification accuracy was 87 percent (Table 30). Samples of age 1+ fish should contain the only significant numbers of hatchery origin fish. Samples sizes were not as large as the 100 per standard normally used as a minimum, so these results should be viewed as preliminary. Again, it is not known at this time how representative age 1+ Dry Creek scale patterns are of Satus system age 1+ patterns in general.

Table 30. Classification accuracies for two age 1+ 3-way steelhead linear discriminant function analyses: A) Dry Creek vs Wapatox vs Roza model and B) Hatchery vs Dry Creek vs pooled Wapatox-Roza model. The hatchery group is composed of Nile Pond and Nelson Spring fish. Eight scale variables were forced into the functions: Focus to T7.

A) .

Correct group	Classification result in percent Dry Creek	Wapatox	Roza
Dry Creek	a4	3	13
Wapatox	a	50	42
Roza	20	40	40

B).

Correct group	Classification result in nercent Dry Creek	Wapatox/Roza	Hatchery
Dry Creek	86	9	5
Wapatox/Roza	12	a2	6
Hatchery	3	4	94

Naturally rearing age 2+ and 3+ samples could not be accurately discriminated using LDF analysis of scale patterns. Three-way age 2+ Wapatox/Roza/Dry Creek and two-way age 3+ Roza/Wapatox model had mean overall classification accuracies of 44 and 47 percent, respectively. Inspection of the age 2+ and 3+ group centroids in Fig.13 shows that they are separated by relatively small distances (mean between-group distances of only 1.0 and 1.3, respectively).

Identifying Steelhead and Resident Rainbow Trout via SPA

Steelhead and resident rainbow trout can be found within the same portion of the Yakima River at times and can be confused when sizes overlap. A method of identifying individuals or estimating the proportion of steelhead and rainbow trout in a sample of fish would be of value.

Scale patterns of known steelhead smolts collected in 1989 from Roza and Wapatox juvenile traps and Dry Creek and ranging in age from 1+ to 3+ (n=242) were pooled and compared to a pooled sample of rainbow trout from Umtanum, Cherry, and Wilson creeks collected in March 1990 ranging in age from 2+ to 5+ (n=44). Classification accuracies between the steelhead and rainbow trout groups were 75 and 73 percent, respectively, based on a LDF analysis using eight scale variables: Focus to T7. Since only eight variables were able to be measured on all fish due to the small number of circuli on scales of smaller fish, it was necessary to limit the analysis to these eight variables. Classification accuracies must be greater

than 90 percent in order to begin to accurately identify individual fish to their respective groups and based on this preliminary analysis, SPA cannot be expected to accurately identify individual fish as rainbow or steelhead trout. However, it does appear possible to estimate the proportion of each group in a mixture given percent classification accuracies in the mid-70's.

Simulation analysis using steelhead and rainbow trout scale measurements was done in order to determine the overall bias and accuracy of the two-way model under a wide range of mixing proportions (see General Simulation Methods in Chinook **Substock** Identification section). Baseline standards representing steelhead and rainbow trout were used to construct 400 mixture samples by randomly selecting 100 samples with replacement from the two standards. Simulation results indicated that overall, based on these particular baseline standards, the proportion of steelhead and rainbow trout in a mixture were estimated with essentially no bias. The mean true composition and the mean estimated composition of steelhead and rainbow trout were equal over the 400 simulated mixtures. Standard deviations for the errors (actual-estimated proportion) were 9 percent for both groups, indicating that approximately 95 percent of the estimates were within 18 percent of the true proportion.

Elemental **Analysis** of Scales

The regression analysis of the number of scales per mg of scale material and fish length showed a negative relationship as expected, given that larger fish require fewer larger, heavier scales to make up one mg of material. The regression line had an r^2 value of 0.36 with a probability level of 0.006, a slope of -0.521 and a y-intercept of 134. A relatively large amount of variation was left unexplained by the relationship, although it did give an indication of the number of scales needed to make up 0.5 mg of material for a fish of a given length. In general, for fish above **140mm** 30 scales should weigh at least 0.5 mg and for fish above **180mm** 20 scales would be required. These values were used as minimum sample sizes for fish within these length intervals.

Elemental analysis of trace elements in juvenile steelhead trout scales will be done as time and funds become available. In addition, more cost effective analytical techniques which are as sensitive as the currently used inductively coupled plasma mass spectrometer are being investigated.

Rainbow Trout Aae **Composition**

A total of 353 **ageable** sc ale samples representing 5 groups of rainbow trout have been aged to date and ages by group are given in Table 31.

Table 31. Age composition in percent for rainbow trout groups collected in 1990. There is some confusion about what tributary sections are represented in these samples due to lack of communication between the WDF's scale and GSI lab. Some samples are listed by internal WDF GSI lab codes (e.g. 90EL) and will be identifies to tributary section as the relevant data are retrieved from archived samples.

Cluster	Group/Section	Date	Percentage of fish by age class						Sample size
			0+	1+	2+	3+	4+	5+	
1	**Umtanum**								
	Section 1	**Sept. 4**	19	77	4	0	0	0	26
	Section 2	Sept. 4	0	a3	17	0	0	0	24
	Section ?	March ?	0	0	0	SO	25	25	16
2	**Cherry Creek**								
	Section 1	Nov. 20	100	0	0	0	0	0	7
	Section 2	**Nov.** 20	3s	33	25	4	0	0	24
	Section 3	Nov. 20	2s	67	**8**	0	0	0	12
	Wilson Creek								
	Section 2	Nov. 21	25	0	25	SO	0	0	4
	Section 3	Nov. 21	0	73	16	9	0	0	11
	Section ?	**March** ?	0	0	7	67	20	7	**15**
3	**Manastash**								
	Section 1	August 16	60	40	0	0	**0**	0	10
	Section 2	**August** 16	10	90	0	0	**0**	0	10
		Sept. 24	22	76	0	0	**0**	0	9
	Section 3	August 16	33	67	0	0	**0**	0	**15**
	Swauk								
	section1	August 20	6	94	0	0	**0**	0	17
	Section 2	**August** 20	20	56	17	0	**0**	0	18
	Section 3	August 20	7	SO	43	0	**0**	0	14
	Taneum								
	Section 1A	**Aug.** 17 and 29	0	100	0	0	**0**	0	1
	Section 2	**Aug.** 17 and 29	0	36	11	0	**0**	0	47
4	**Midddle** Fork **Teanaway**								
	Section 1	Sept 10 and 17	32	**55**	14	0	**0**	0	22
	Section 2	**Sept** 10 and 17	33	33	33	0	**0**	0	21
	WF Teanawy								
	Section 1	**Aug** 3, **Sept** 6	67	20	13	0	**0**	0	**15**
	Section 2	**Aug 3,** Sept 6	33	SO	**8**	**8**	**0**	0	12
	Section 3	**Aug** 3, **Sept** 6	33	SO	17	**0**	**0**	0	12
	Section ?	**Aug** 3, Sept 6	0	40	SO	**10**	**0**	0	10
	NF Teanawy								
	Section 2	Sept. **5** and 6	14	47	39	**0**	**0**	0	49
	Mainstem Yakima								
5	**Section** 1	Sept. 20, Oct. 9 and 22	0	**45**	45	**0**	0	9	11
6	**Section** 3	Nov. **8**	0	**58**	33	**8**	0	0	12
7	**Section** 4	Oct. 15 and 23, **Nov. 8**	10	SO	40	**0**	0	0	10
	Section 5	Feb. 21	0	0	**18**	**64**	9	9	11
		Oct. 24 and 25	0	42	42	**8**	**8**	0	12
8	Section 6	Feb. 21	0	0	0	SO	SO	0	2
		Sept. 20, Oct. 23, Nov. 11	0	44	36	**8**	**8**	0	13
	Section 7	Sept. 20 **and** 27, Oct. 4	0	57	43	**0**	**0**	0	7
??	**Section ?**	**Sept.** 11	2	53	37	**3**	**3**	2	60

Freezing Effects on Body Length Measurements

Backcalculation analyses and size-at-age distributions require accurate length measurements of fish at the time of capture. At times it is not possible to take length measurements at capture and fish are frozen and later thawed and measured. In order to determine the amount of body shrinkage that occurs while samples are stored in freezers, three groups of fish were measured for fork length just after capture and then frozen. One pooled group of rainbow trout (n=28) and Wapatox steelhead (n=39) ranging in length from 67 to 340 mm, and two groups of steelhead smolts from Satus Creek (n=64, range 66 mm to 190 mm), and Logy Creek (n=88, range 66 mm to 193 mm) were analyzed using least-squares linear regression analysis. After freezing for about 3-5 months, samples were thawed at the WDF GSI lab and fork lengths (tip of snout to fork of tail) were again measured. Length-at-capture (LAC) was then regressed against length-after-thawing (LAT). Regression results for the three groups were very similar. Each regression model explained nearly all of the sample variation (r^2's ranged from 0.97 to 0.99; Fig. 14 gives an example). No y-intercept was significantly different than zero (PL0.22). All slopes were significant (P<0.001) and ranged from 1.026 to 1.042, indicating a shrinkage of about 3 to 4 percent in fork length during 3-5 months storage in a freezer.

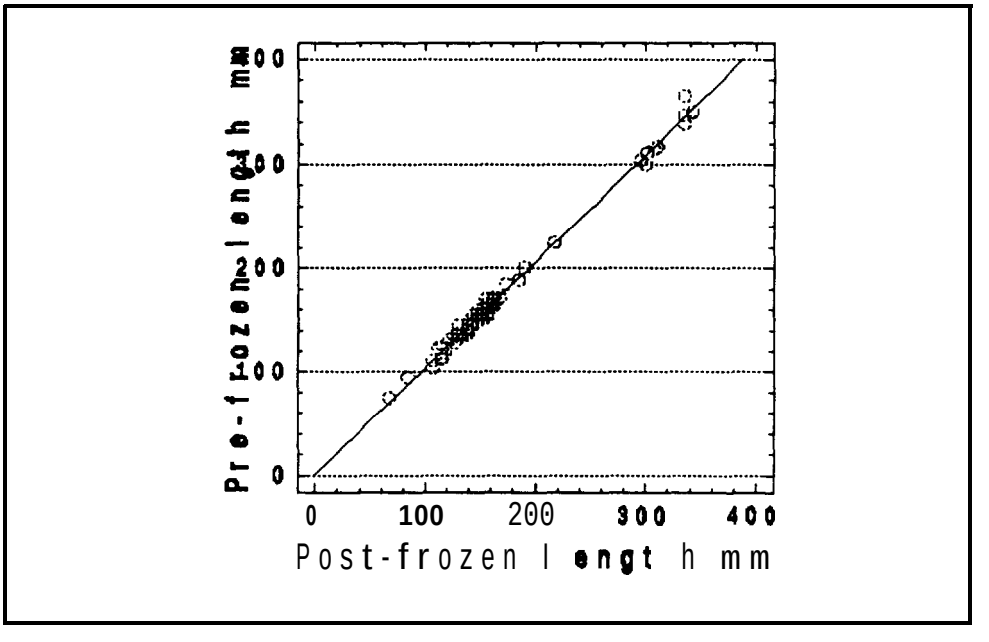

Fig.14. Least-squares linear regression analysis of pre-frozen and post-frozen body length (mm) of rainbow (n=28) and Wapatox steelhead (n=39) trout.

SUMMARY RECOMMENDATIONS

♦ Continue to scale sample outmigrants at Wapatox, Roza, Prosser, Satus and Logy creeks to determine interannual and between site variation in age composition and length-at-age. Scale samples should also be taken from any additional smolt populations targeted for GSI sampling. Replicated sampling over a number of years is necessary for estimating the inter-annual variation in smolt age composition and length-at-age.

♦ Establish methods to scale sample adult steelhead returns at Prosser and the mouth of the Satus system as they pass upstream in order to more accurately estimate adult age at return and size-at-age. A comparison of the two samples would help determine if demographic differences exist between the Satus Creek system and other upper river steelhead stocks. Scale samples collected from kelts are not as useful since kelts are not representative samples of the spawning population. This is because steelhead do not survive spawning and move back downstream in a random manner. Rather, post-spawning larger older fish die at lower rates than smaller younger fish which skews the age distribution of kelts toward older larger fish.

♦ Collect otoliths from adult steelhead kelts and mature rainbow trout and use a trace element microprobe to determine the concentration of strontium in the otoliths' nuclei. Fish with elevated levels of strontium are the progeny of anadromous females (Ralish 1990). Fish with low levels of strontium are the progeny of non-anadromous females. This occurs because anadromous females absorb strontium at relatively high levels from saltwater while their eggs are developing and the strontium gets absorbed into their eggs. Once fertilization occurs, developing embryos incorporate available strontium into the first developing hard part: the otolith nucleus. If this technique is successful, the proportion of steelhead and rainbow trout adults produced by anadromous and non-anadromous females could be estimated from representative adult otolith samples. Reference samples of eggs and progeny of known anadromous and non-anadromous females should be collected and analyzed to determine the accuracy of the technique.

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REPORT NO. 2

STOCK IDENTIFICATION MONITORING TOOLS

- Fluorescent and Elemental Marking of Juvenile Chinook Calcified Tissues.
  by Curtis M. Knudsen, Steve Schroder, Mark Carr, and Gene Sanborn

- Evaluation of Tagging and Marking Techniques For Use in the Yakima/Klickitat Fisheries Project
  by Curtis M. Knudsen

FLUORESCENT AND ELEMENTAL MARKING OF JUVENILE CHINOOK SALMON CALCIFIED TISSUES

INTRODUCTION

As part of WDF's ongoing research into stock identification and marking techniques, we are evaluating mass marking techniques in order to determine their effectiveness in measuring such critical response variables as post-release survival of experimental and control groups and reproductive success. An additional objective is to identify marking methods that permit benign identification of hatchery and natural adult returns during broodstock collection. That is, adult hatchery returns must be identified and released unharmed in order to supplement the natural spawning population. In the context of the YKFP, an effective mass marking technique must necessarily fulfill the first four of the following needs and, in order to be a practical tool suitable for long term use, must meet the latter two needs, as well:

1. Entire populations must be unequivocally marked with no significant decrease in short term fitness due to the marking process or mark presence, and no significant decrease in long term fitness due to effects on physiological competence, migration patterns/timing (down or upstream), or predator avoidance. Also, minimum size requirements for tag application must not exclude currently scheduled size-at-release in the YKPP.

2. The mark must be benignly recoverable. That is, once a fish has been sampled for the presence of a mark, the fish must still be alive and able to resume its migration with minimal impact to its survival and ultimate reproductive success.

3. More than one mark code is necessary. For example, at least 15 unique codes are required to identify the experimental and control releases of spring chinook each year in the initial phases of the current YKFP experimental design.

4. The mark should be recoverable from juveniles, upstream migrating adults, spawning adults, and carcasses without bias (no significant tag loss over time).

5. The mark should be economically and logistically practical in terms of mark application, sampling requirements for juvenile and adult fish, and analytical/detection requirements for mark decoding.

6. Marked fish should be identifiable within 48 hours or less after being sampled. Initial generic identification of fish of hatchery and natural origin should be made within this time frame in order to minimize impacts on upstream migration. Identification of specific release group codes can take place over a longer time period.

No marking technique currently being used fulfills all six of these requirements. One marking technique that has the potential to meet these needs is marking mineralized tissues, particularly scales, using a combination of trace elements and fluorescent compounds. Fluorescent compounds or fluorochromes such as tetracycline, alizarin complexone, xylenol orange, and calcein (fluorescein) have been used to mark mineralized tissues of mammals and fish (Hankin 1978, Rahn and Perren 1970, Tsukamoto et al. 1989, Weber and Ridgway 1962). These compounds bind with alkaline earth metals, such as calcium in bone and scales, and fluorescence under ultraviolet light in distinct colors. Trace elemental marking of fish has successfully been demonstrated in coho salmon (Behrens Yamada and Mulligan 1982, 1990; Brown 1991), adult chum salmon (Kato 1985) and fry (S. Schroder, WDF, 1991, pers. comm.), sockeye salmon fry (S. Schroder, WDF, 1991, pers. comm.), and striped bass (c. Coutant, Oakridge National Laboratory, 1991, pers. comm.) using either immersion in a solution of the marking material or feeding element enriched diets. In addition, naturally occurring differences in concentrations of trace elements in scales have been used to identify wild stocks of sockeye salmon (Lapi and Mulligan 1981).

The advantages of fluorescent and elemental marks are:

1. Mass marking of entire populations is possible at a relatively early age by incorporating the marking compounds into feed or immersing fish in a marking solution. Potentially, fish could be marked as soon as they begin to form scales, beginning at about 35-40 mm in salmonids.

2. Fluorescent mark decoding is cheap, simple, and can be done on a real time basis. While the epi-fluorescence microscopy equipment used is more sophisticated than that found in a typical lab, it is not extremely expensive (<$15,000), does not require a highly trained specialist to operate or calibrate, requires only visual recognition of a mark, and could be set up in the field at an adult monitoring facility if necessary. Perhaps the biggest challenge in elemental marking is to develop and refine microanalytical techniques that can identify spatially separated bands of concentrated elements and to make this analytical technology available to fisheries agencies for rapid turn around in analysis. The technology currently being used only allows analysis of whole tissue samples. This results in dilution of the marking

element which is typically concentrated in a narrow band of calcified material. The analytical lab currently being used by WDF and Canadian Department of Fisheries and Oceans for elemental analysis of hard parts is located in Vancouver, Canada making 48 hour turnaround time in analysis results questionable. However, by using a single fluorescent mark as a generic visible mark, the equivalent of the adipose fin-clip, this problem can be managed (see 4. below). Microanalytic techniques focus the elemental analysis onto a narrow area of the hard part 20-30 microns in diameter, which minimizes dilution effects and allows trace elements to be detected with much greater sensitivity. In addition, the location of the mark on the calcified structure can then be determined and this information used to create unique codes based on mark placement within the calcified structure.

3. Marks can be detected benignly by removing and analyzing a piece of mineralized tissue. Scales are the easiest and least invasive calcified structure to remove. However, other easily removable calcified structures such as fin rays and opercle punches should be explored, as well, should scales prove to be ineffective.

4. A generic fluorescent mark can be used as a flag or 'external' mark for elementally marked fish. Just as all coded-wire tagged fish are adipose fin-clipped, all elementally marked fish could have their scales fluorescently marked. Thus, a returning hatchery adult with a fluorescent mark can be quickly identified, sorted from the unmarked naturally produced fish, and allowed to continue its upstream migration. Decisions can then be made on broodstock selection from the unmarked natural fish. The elemental mark can be decoded at a later time from the same scale sample used for epi-fluorescent analysis. A fluorescent mark allows quick visual identification of unmarked fish and should dramatically reduce the number of scales which must be processed for elemental mark decoding. This in turn will reduce analytical costs and effort significantly. Thus, the development of a single successful fluorescent marking compound will have significance. At a minimum, such a mark will allow supplemented fish to be generically marked and subsequently identified benignly at little cost and in real time.

5. Once applied, for a mark to be acceptable in the context of the YKPP, it should be detectable throughout the life of a fish and the group code should be recoverable more than once. Hankin (1978) states that calcein marks on guppy scales were visible for at least 3 months. However, it appears that he did not monitor fish for mark retention beyond 3 months. No information currently exists on how successful xyienol orange or alizarin may be as salmonid mass marking compounds. The elements strontium (Behrens-Yamada and Mulligan 1990),

lanthanum and samarium (B. Enovar, UBC, 1991, pers. comm.) and europium (C. Coutant, Oakridge National Lab, 1991, pers. comm.) have been shown to be stable in the calcium matrix of scales, once they are incorporated. Only strontium marked coho have been monitored for marks on live fish for any extended time (18 months) and the mark was detectable on adult returns (Behrens-Yamada and Mulligan 1990).

6. Unique codes are possible by using different elements or colored fluorochromes together or alone (Rahn and Perren 1962, Olerud and Lorenzi 1970, Suzuki and Mathews 1970), by marking fish at different sizes (Hankin 1978), thus placing marks at different locations within the mineralized structure, and by using different concentrations of the same element. While the number of unique codes may not reach the number possible with binary coded wire tags, for many purposes the number of codes should be sufficient to carry out detailed studies where multiple experimental and control groups are necessary. Without the ability to determine mark location within the calcified structure, four elements used alone and in combination are needed to create 15 unique codes. If the location of distinct marking bands can be determined and multiple elements are detectable within each band then two bands and four elements will give 255 unique codes. This demonstrates the value of having spatial information on mark placement.

Some of the compounds cited above have not been tested on fish or tried on large populations for mass marking purposes. Thus, much of the initial work to be performed is of a basic nature addressing such issues as mortality and marking success of different compounds and elements at various dosages and feeding durations. Fluorescein and tetracycline compounds have been used to successfully mark scales of guppy (Hankin 1978) and salmon (Weber and Ridgway 1962), respectively, although tetracycline marks on scales were not visible after a few days due to photobleaching. Alizarin complexone and calcein have been used to mass mark the otoliths of juvenile marine species (Beckman et al. 1990, Tsukamoto et al. 1989, Wilson et al. 1987), but have not been used to mark the scales of salmonids. Xylenol orange has not been used on any fish species as a marking compound.

In Part I of this study we will feed the fluorochromes xylenol orange, calcein, and alizarin to chinook salmon at various dosages and durations with the intent of producing a visible mark on calcified structures, especially scales, when examined using epi-fluorescent microscopy and to determine if significant mortality occurs due to ingestion of any enriched diets. In addition, the rare earth element cerium will be fed in an attempt to produce a band of scale material with significantly elevated cerium levels. The initial phase should be considered a feasibility study; a

chance to place all the pieces together and work out operational problems. Part II will build on the knowledge gained in Part I and further examine feeding fluorochromes which produced significant results in Part I, changing dosage or duration as needed. In addition, diets enriched with samarium, lanthanum, strontium and cerium will be tested. Finally, short term (24 hour) immersion of fish in concentrated baths will be investigated as a method of introducing samarium, lanthanum, strontium and cerium into the scales and other calcified parts of chinook.

PART I: APRIL 8 TO MAY 20, 1991

Methods

On April 5, 1991 25,600 chinook were taken from the main production pond (Pond 9) at George Adams Hatchery, randomly divided into lots of 400 fish, and placed into 2.5 foot circular continuous flow self cleaning tanks. The fish were allowed to acclimate unfed before treatments began three days later. A sample of 187 fish from Pond 9 had mean fork length of 52 mm (sd= 6.7 mm, range 40-69 mm; **Fig.15)** and weight of **1.4 g** (sd= 0.6 g, range 0.5-3.2 g; **Fig.16).** The population in Pond 9 was the product of **13** separate egg collections taken over a 43 day period and this resulted in relatively large variation in initial lengths and weights.

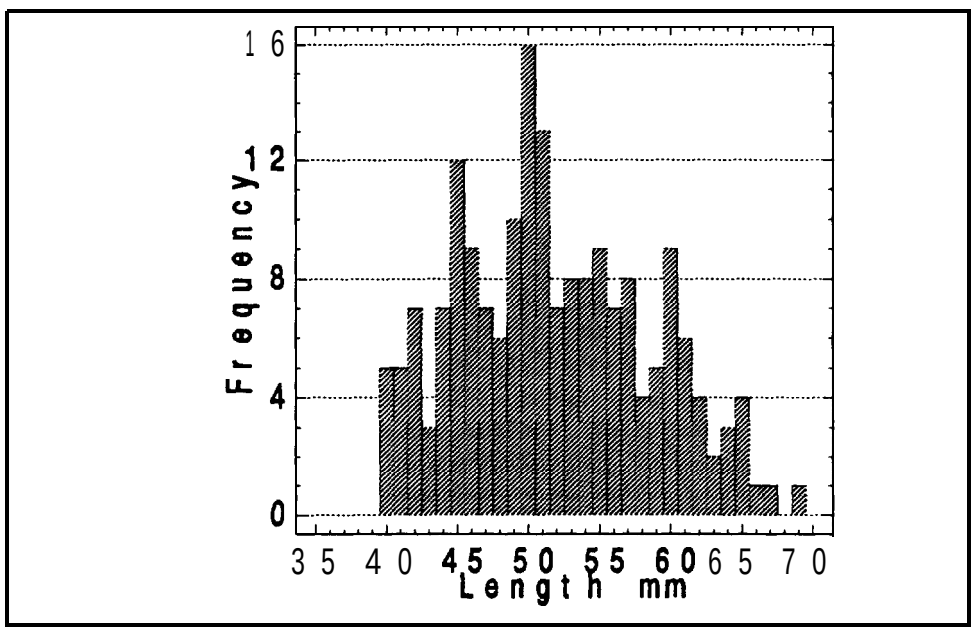

Fig.15. Length frequency distribution of George Adams chinook on **4/5/91** just prior to beginning treatments.

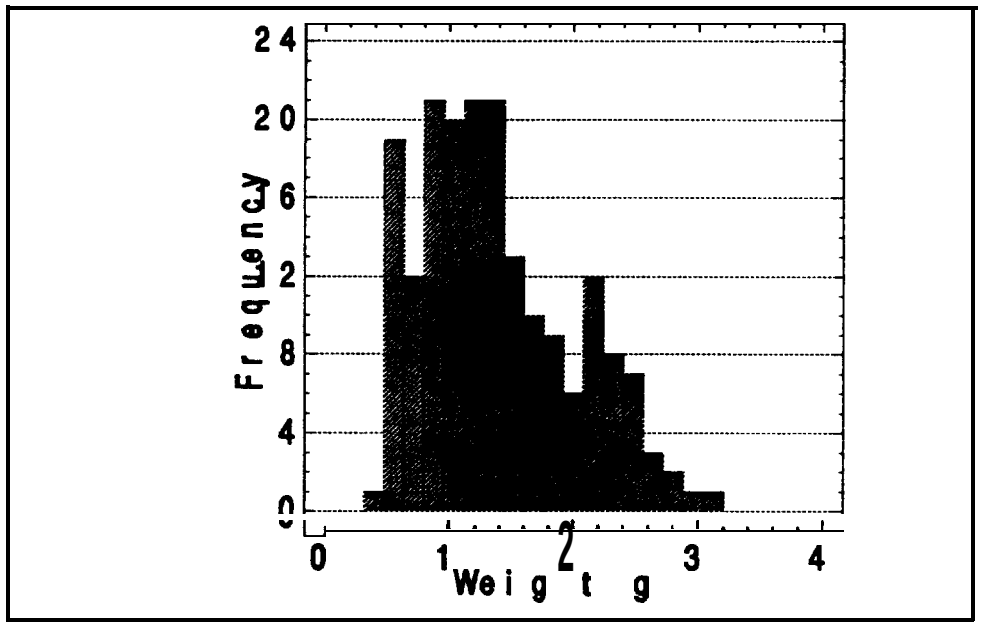

Fig.16. Weight frequency distribution of George Adams chinook on **4/5/91** prior to beginning treatments.

Treatment diets were enriched with four compounds: calcein, xylenol orange, alizarin red, and **cerium.** A low calcium diet was manufactured at NMFS Montlake lab in cooperation with Ron Hardy. Marking compounds were dissolved in demineralized hake viscera and added to AHS low calcium salmon meal (Moore-Clark) with cellulose filler, wheat bulk and vitamin C. A low calcium diet was used to reduce any interference that might occur due to calcium competing with the marking compounds as they were incorporated into calcified tissues. The treated feed was then processed through a **1\16** inch diameter die and cut into approximately **1/8** inch pellets. This size pellet was used based on an anticipated mean fish length of 60 mm. However, due to the smaller size and large variation in fish length at the beginning of the experiment, many fish were less than 60 mm and the pellet size of the experimental diet was too large for the smallest fish to easily consume. Consequently, marking success in smaller fish was likely reduced.

Each compound was fed at 6 dosage levels and over either 8 or 16 days and two low calcium and two normal control diets were included per compound (Table 32). This resulted in a total of 64 groups (48 treatments and 16 controls).

Table 32. Initial population size per tank for treatment groups by dosage (grams of marking material per kg low calcium diet) and feeding duration for cerium, xylenol orange, calcein, and alizarin, as well as, low calcium and normal OMP control diet groups.

	Dosaae (a compound/kg low Ca diet)						Controls	
Duration	0.10	0.25	0.50	1.00	2.00	4.00	Low Ca	Normal Ca
8 days	400	400	400	400	400	400	400	400
16 days	400	400	400	400	400	400	400	400

Fish were fed control and treatment diets ad libitum. If fish were not actively taking feed no additional food was given and excess food was not allowed to accumulate for extended periods on the bottom of tanks. Tanks were monitored for mortality and 10 fish samples were periodically collected, sacrificed and stored frozen. Scale samples for initial epi-fluorescent analysis were collected from selected groups of the periodic samples. The scales were soaked in water for a few minutes, mounted under cover slips in glycerin, and viewed with a Nikon microscope equipped with a high pressure 50 watt mercury lamp (Nikon EF-D Mercury Set) and Nikon EX400-440 exciter and BA470 barrier filters at 10 and 20x. The groups with the greatest likelihood of being successfully marked were selected for initial screening for marks. These included the two highest dosage and longest duration groups (2 and 4 g/kg for 16 days) of cerium, alizarin red, and xylenol orange and the two lowest dosage and longest duration groups for calcein (0.1 and 0.25 g/kg for 16 days).

Groups in which all fish were not marked after the end of the monitoring period or which exhibited significant mortality were sacrificed. Food and Drug Administration restrictions do not allow releases of fish fed any treatment diet. At the end of the monitoring period random samples of forty fish were collected from each tank, length measurements taken and the fish stored frozen. Control groups were released with the normal hatchery production after 40 fish samples were collected.

Other calcified parts such as otoliths, vertebrae, opercula, and fin rays will be analyzed as time and resources allow.

Recent work at Oakridge National Laboratory (ONL) has shown that previously undetectable concentrations of the rare earth element samarium fed at a dosage of approximately 1 g\kg feed in striped bass (Muncy et al. 1988) was detectable using a newly developed laser microprobe (C. Coutant, ONL, 1991, pers. comm.). Scale samples (n=10 fish per group) from the highest and lowest dosage 16 day cerium treatments and a normal calcium control group will be sent to the ONL for analysis of elevated levels of cerium. These three treatment groups along with the 8 day duration 0.10 and 4.00 g/kg cerium and low calcium control groups will continue to be

reared at George Adams Hatchery until results from Oakridge are received. Should the cerium groups have an identifiable mark, they will continue to be reared through the fresh and saltwater phase of their lives to determine mark longevity.

Preliminary Part I Results

The following preliminary results on mark detection refers to the ten fish periodic samples which were collected and should be treated as qualitative rather than quantitative results. Analysis of the final 40 fish samples from each tank have not been completed yet.

A fluorescing mark was detectable on the two lowest concentrations of calcein in some fish, although it was not a concentrated band of color as was anticipated, but rather a diffuse green/blue color throughout the scale. Since the mark did not appear as a bright band, it was not clearly identifiable until control scales were examined adjacent to the treated scales. Four calcein groups (0.10 and 0.25 g/kg and 8 and 16 day durations) will be reared further in order to track the marks presence over a longer time period. In Part II, calcein treatments will be extended to 24 days in an effort to increase the strength of the mark. Calcein dosage levels cannot be significantly increased beyond 0.25 g/kg due to the aversion fish showed for dosages greater than 0.25 g/kg feed and the consequent mortality from starvation.

Xylenol orange produced a fluorescing mark on scales of some, though not all, fish in the 4 and 2 g/kg 16 day groups. The mark appeared as a faint reddish/orange band on the scale 1 to 2 circuli wide. Mark intensity was positively related to dosage level. However, mark intensity faded within days after scales were collected and mounted on slides. Since not all fish were marked and mark intensity was faint, these groups were not reared further. Xylenol orange dosage levels will be increased in Part II below in order to create a more distinct and longer lasting mark, since fish experienced no significant mortality or aversion to the feed at the 4 g/kg dosage. In addition, the reason for the mark's fading will be investigated in order to determine if changes in feed handling, sample collection or preparation can reduce fading.

No alizarin or ceriumtreatmentgroups had a detectable fluorescent mark on scales. Elemental analysis of cerium groups has not been completed at this time.

Calcein dosages equal to or greater than 0.50 g/kg were not palatable and dosages above 1.0 g/kg were rejected by fish over the entire 8 and 16 day treatment periods. Although fish would strike the first few pellets dropped into a tank no feed in dosages greater than 1.0 g/ kg feed was actually observed being consumed.

All other treatment diets (cerium, xylenol orange and alizarin) appeared to be consumed by fish equally well.

Mortality within tanks ranged from 0 to 33 fish (Table 33) over 46 days. Bootstrap computer simulation analysis was used to analyze the mortality data (see Noreen 1989 for a discussion of this technique). The null hypothesis was that each treatment group's mortality was **equal** to or less than the control groups' (a **one-**sided test). The alternative hypothesis was that mortality of a treatment group was greater than the control groups. Mortality data for the eight low calcium control. groups (mean 2.9 mortalities) and eight normal diet control groups (mean = 3.4 mortalities) were pooled into a single vector of zeros representing live fish, and ones representing mortalities. A computer program was written to sample this vector 200 times with replacement and calculate the number of resulting **"mortalities"** in the 200 fish sample. The bootstrap sampling procedure was repeated 5,000 times and the resulting mortality frequency distribution **(Fig.17)** was used to construct an empirical significance test. The probability of 7 or more mortalities occurring, based on the frequency distribution in **Fig.17,** was 4 percent or less. Thus, any treatment group with 7 or more mortalities had significantly higher mortality than the control groups at the alpha equals 0.04 level. Significant treatment groups are indicated by an asterisk in Table 33. Most of the treatments with significant mortality (78 percent) occurred in the calcein groups. Only 3 significant differences were found in the 36 cerium, alizarin and xylenol orange treatments. Two significant differences in 36 tests would be expected due to random chance alone. It is speculated that the almost total aversion fish showed for the higher calcein treatments contributed significantly toward higher mortality through starvation. Most dead fish were much smaller than average and emaciated; resembling **"pin-heads".** Mortality was generally spread out over the entire 46 days and not concentrated in the days just before and after treatments ended indicating that if there were toxic affects they were not strong and immediate in effect.

Table 33. Total mortality for the 64 experimental and control groups of chinook over 46 days. Groups with significantly higher mortality (alpha=0.04) than the control groups are indicated by an asterisk.

Group	Mortality (number of fish)
Controls	
Normal diet Rap 1	5
Normal diet Rep 2	3
Normal diet Rap 3	2
Normal diet Rap 4	4
Normal diet Rep 5	3
Normal diat Rep 6	4
Normal diet Rep 7	3
Normal diet Rap 8	3
Lou Calcium Rep 1	2
Lou Calcium Rap 2	5
Lou Calcium Rap 3	4
Lou Calcium Rep 4	6
Low Calcium Rep 5	1
Low Calcium Rep 6	4
Low Calcium Rep 7	0
Low Calcium Rep 8	1
Treatments	
Calcein 0.10 8 days	4
Calcein 0.25 8 days	10*
Calcein 0.50 8 days	11*
Calcein 1.00 8 days	24*
Calcein 2.00 8 days	4
Calcein 4.00 8 days	14*
Calcein 0.10 16 days	7*
Calcein 0.25 16 days	a*
Calcein 0.50 16 days	33*
Calcein 1.00 16 days	14'
Calcein 2.00 16 days	18*
Calcein 4.00 16 days	33*
X. orange 0.10 8 days	3
X. orange 0.25 8 days	6
X. orange 0.50 6 days	5
X. orange 1.00 6 days	4
X. orange 2.00 8 days	1
X. orange 4.00 6 days	4
X. orange 0.10 16 days	1
X. orange 0.25 16 days	3
X. orange 0.50 16 days	4
X. orange 1.00 16 days	11*
X. orange 2.00 16 days	5
X. orange 4.00 16 days	6
Alizarin 0.10 8 days	3
Alizarin 0.25 8 days	1
Alizarin 0.50 6 days	2
Alizarin 1.00 8 days	3
Alizarin 2.00 8 days	6
Alizarin 4.00 8 days	3
Alizarin 0.10 16 days	5
Alizarin 0.25 16 days	3
Alizarin 0.50 16 days	2
Alizarin 1.00 16 days	4
Alizarin 2.00 16 days	6
Alizarin 4.00 16 days	4
Cerium 0.10 8 days	7*
Cerium 0.25 6 days	4
Cerium 0.50 8 days	1
Cerium 1.00 6 days	3
Cerium 2.00 8 days	2
Cerium 4.00 8 days	2
Cerium 0.10 16 days	4
Cerium 0.25 16 days	5
Cerium 0.50 16 days	a*
Cerium 1.00 16 days	6
Cerium 2.00 16 days	2
Cerium 4.00 16 days	2

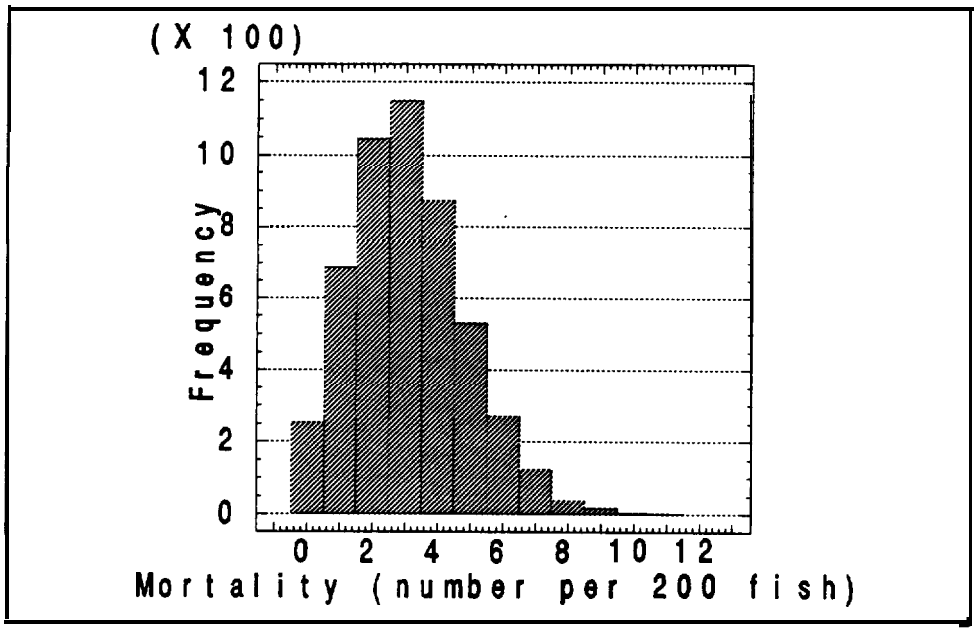

Fig. 17. Frequency distribution of 5,000 bootstrapped samples (n=200 fish per sample) taken from the pooled control groups mortality data. X-axis is number of mortalities per 200 fish over 46 days.

PART II: JULY 8 TO AUGUST 30

Methods

Chinook from the McKernon Hatchery (WDF) were placed into rearing tanks located at George Adams Hatchery on July 7, 1991. Samples of 20 fish per tank were anesthetized and fork length and weight measured. After recovering from the anesthetic fish were placed back into their respective tanks. Fish will be reared in the circular tanks used in Part I at 100 fish per tank for the fed groups'and 50 fish per tank for the immersion groups.

The fluorescent compounds calcein and xylenol orange, and the elements cerium, samarium, lanthanum and strontium will be fed at various dosages and in combination for either 16 or 24 days (Table 34). The experimental diet groups will occupy a total of 36 tanks with control groups occupying an additional 4 tanks. Feeding treatments began July 8, 1991. There are also 24 tanks with 50 fish per tank to be used for testing immersion in baths of the elements cerium, samarium, lanthanum and strontium for 24 hours. Immersion experiments are scheduled to begin between July 24 and July 30, as time and manpower allow.

Table 34. Dosage and treatment durations for Part II feeding study. The elements cerium, strontium, lanthanum, and samarium and the fluorochromes calcein and xylenol orange will be fed.

Markina compounds	Dosase (g/kg feed)	Duration (days)
Calcein	0.05	24
Calcein	0.10	24
Calcein	0.20	24
Xylenol orange	5.00	16
Xylenol orange	10.00	16
Xylenol orange	15.00	16
Cerium	5.00	16
Cerium	10.00	16
Cerium	15.00	16
Lanthanum	5.00	16
Lanthanum	10.00	16
Lanthanum	15.00	16
Samarium	5.00	16
Samarium	10.00	16
Samarium	15.00	16
Strontium/Lanthanum	2.00/2.00	16
Strontium/Samarium	2.00/2.00	16
Strontium/Cerium	2.00/2.00	16

In Part I, feed rations were allowed to remain in direct sunlight on the top of tanks for up to 7 hours in some cases. This likely caused the fluorochromes to photobleach to some unknown extent reducing the compounds ability to fluoresce under ultra-violet light. Fish feed will be stored in opaque containers in order to eliminate exposure to direct sunlight in Part II.

Fed groups will be sampled (n=40 fish per population) 28 days after treatments have ended. Scales will be collected and analyzed for either a fluorescent or elemental mark, depending on the marking treatment. Any group in which all fish have been marked and which experience no significant increase in mortality will continue to be reared in order to monitor mark persistence.

Toxicity of elemental baths of cerium, samarium, lanthanum, and strontium will be determined by exposing 5 fish lots to increasing concentrations of each element for 24 hours. Once a maximum (non-toxic) dosage has been established, groups of fish will be immersed in baths of increasing strength up to the maximum dosage for 24 hours and monitored for mortality for 30 days. At the end of 30 days the fish will be sacrificed and stored frozen. Scale samples will be collected for analysis using both inductively coupled plasma (ICP) mass spectrometry and laser microprobe analysis. Carcasses will be saved for further hard part analysis.

SUMMARY RECOMMENDATIONS

♦ Continue Part II of the above study at George Adams Hatchery. Analyze the elemental treatment samples taken for both Part I and II using both ICP mass spectrometry and laser microprobe analysis to determine whether detectable marks are present and, if so, which technique will give the most accurate identification of marks. Explore methods of sample handling and processing which will enhance and clarify fluorescent marks. Identify and test fluorescent mark potentiators, such as glucosamine hydrochloride, and determine if they can enhance the marking ability of xylenol orange and calcein. Continue to rear fish successfully marked in order to monitor the mark's longevity through maturation. This will involve marking fish by treatment group and holding fish for long term rearing in saltwater net pens. Microanalytical methods will be developed in cooperation with **Oakridge** National Laboratory. In addition, we will begin researching the requirements necessary to secure FDA approval for use of any elements which successfully mark fish. We will coordinate our efforts with Canada Department of Fisheries and Oceans, **Oakridge** National Laboratory, and CRITFC personnel working on these same problems.

♦ Begin work on developing the methodology to mark progeny of gravid females using elemental marks on otoliths. This would include strontium, lanthanum, **cerium,** and samarium. Begin by conducting controlled small scale experiments using fertilized eggs immersed in increasing concentrations of marking compounds and untreated control groups. Test for effects on egg viability, survival, developmental abnormalities, and marking success by elemental concentration and compound. From this information estimate dosage concentrations to administer to gravid females. The technique should then be applied to a small number of adult females by injecting gravid females with solutions of marking materials. The females would then be artificially spawned and measurements made of **egg** viability, survival, developmental abnormalities and marking success. This initial work will likely result in significant mortality for some egg lots and females. Therefore, it is recommended that these preliminary tests be conducted on a population of hatchery chinook salmon from outside of the Yakima system in order to eliminate the risk to Yakima natural substocks.

♦ Continue to develop elemental marking methods directed at identification of experimental and control groups of supplementation fish and characterizing differences between natural substocks. This will include development of an efficient method of introducing elemental marks (immersion or feeding) into chinook, determining dosage level, and refinement of microanalytical methodologies.

♦ Temporal water quality samples taken by Bureau of Reclamation from throughout the Yakima River system over the past 2 to 3 years will be examined to identify differences in the water chemistry between the upper and lower river. Significant differences in the concentrations of elements and compounds in the water at these sites would indicate that there could be significant differences in the chemical composition of the hard parts of fish which reared in the lower river (early outmigrants) and upper river (yearling outmigrants). A simple controlled experiment could be performed determine if such differences in hard part chemical composition can occur by comparing the chemical composition of hard parts from two group-s of 100 fish reared in the lower river which were collected in November/December as they moved downstream at Wapatox and Roza to the composition of the hard parts of two groups of 50 fish collected as yearling outmigrants in the spring at Wapatox and Roza. Microprobe analysis of the two "early migrating" groups and the two "late migrating" groups' scales and otoliths will be performed,.' Comparison of the groups' chemical compositions will determine if significant differences have occurred and where within the hard parts suchdifferences occur. If significant differences are found, then the accuracy of separating early and late migrants will be estimated using linear discriminant and maximum likelihood estimation methods. Should the accuracy be sufficiently high, this method could be used to separate adult returns sampled on the spawning grounds into late and early outmigrants based on the chemical composition of the freshwater portion of the scale and the contribution of each life history strategy to the overall production of each spring chinook substock estimated.

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EVALUATION OF TAGGING AND MARKING TECHNIQUE8 FOR USE IN THE YAKIMA/KLICKITAT FISHERIES PROJECT

INTRODUCTION

In order to test the hypothesis that new artificial production in the Yakima and Xlickitat sub-basins can be used to increase harvest and enhance natural production without negatively impacting the already existing genetic resources, an experimental program is being implemented (EDWG 1989). In the course of implementation, it will be necessary to evaluate various stock/group identification methods for use as monitoring tools to measuring critical response variables identified in the Experimental Work Plan. Four broad categories of investigation have been identified: post-release survival, reproductive success, long-term fitness, and species interactions. In addition to identifying tagging/marking methods to measure and monitor response variables related to these areas, techniques must be identified or developed which will facilitate broodstock selection under the unique and rather restricting requirement of benign or nonlethal identification of all hatchery returns once supplementation begins. This is necessary because returning hatchery fish must be excluded from broodstock selection, released and allowed to spawn naturally in order for meaningful supplementation to occur. One marking topic not discussed in great detail is marking/tagging techniques for mark/recapture studies. Only variations on jaw tags are discussed in that context.

One of the principle assumptions made in any marking/tagging study is that the tag or mark and respective application process do not cause significant negative affects on the fish's survival or behavior. Recent very preliminary work by Maynard et al. (1990) found that even those tags or marks generally believed to be least intrusive: CWT, freeze brand and PIT tag, were associated with twice the rate of predation (20-22 percent) on subyearling steelhead by yearling steelhead 2-6 days after marking than unmarked control fish (10 percent predation). Although this study is by no means definitive, such information makes one pause to soberly consider the fact that all supplemented fish must be marked in some fashion once supplementation begins and the chosen marking method should minimize any reductions in the fish's overall fitness.

A preliminary estimate that at least 35 recoveries per replicate group are required in order to detect a 50 percent difference in treatment and control groups has been made based on work by De Libero (1986). For the purposes of the present discussion 35 recoveries will be used as a minimum number of recoveries per replicate and control groups at the particular targeted life history stage. The subject of statistical power necessary to evaluate treatments deserves further consideration and discussion.

Monitoring needs would therefore be the ability to sample at least 35 marked fish per release group at the particular targeted life history stage, e.g. adult return to Horn Rapids. In the Yakima system, the primary concern will be tagging enough juveniles to insure an adequate number of adults are recovered for accurate estimates to be made. For hatchery spring chinook with survival rates of approximately 0.1 percent, this means tagging all fish within any release group of 75,000 and recovering nearly 50 percent of all adult returns from that release group to ensure 35 recoveries are made.

In order to measure components of some of the identified response variables marked adult fish must be recovered on the spawning grounds. Tissue samples, age, sex, length, gamete retention and perhaps fecundity data will be collected from spawning ground recoveries, as well. There are two generic concerns regarding the recovery of adult fish on the spawning grounds (both live and carcass recoveries): 1) typically the proportion of fish recovered on the spawning grounds is a very small fraction of the population, even with very intensive sampling effort (Clutter and Whitesel 1956; Eames and **Hino** 1981) and therefore the number of tagged fish returning must be large enough to insure sufficient numbers of tags are recovered to make statistically valid estimates, and 2) in many cases spawning ground recoveries are biased due to over representation of both females and larger, older fish (Clutter and Whitesel 1956; Eames and **Hino** 1981; Eames et al. 1981; Peterson 1954; Ward 1959; Conrad 1990). This bias is due primarily to females remaining near and continually returning to redds even after being disturbed by recovery operations; females holding and dying in shallower areas than males; and larger fish generally being captured more easily (better **"targets"**) while also being swept out of survey areas or removed from the immediate stream area by predators at lower rates. Any response variable requiring spawning ground recoveries could be effected by these problems. This would include estimating parameters such as straying rates, spawner spatial and temporal distributions, survival rates back to spawning grounds, and monitoring tributaries for genetic and demographics characteristics (i.e. age, sex, length, fecundity, and mean age of female reproduction).

Monitoring at adult passage facilities can provide unbiased samples of adult returns to relatively large portions of the Yakima River, e.g. the **Naches** system. However, it is clear that even existing facilities, e.g. Prosser and Cowiche, can give biased samples because fish do not select passage routes randomly either choosing ladders or jumping dams. In addition, large adult passage facilities do not provide recovery data on a fine enough resolution to estimate straying rates, temporal/spatial spawning distribution, survival rates, or demographics on a **substock** basis unless a very accurate method of identifying individual fish from each natural **substock** is developed. Genetic stock identification (GSI) and scale pattern analysis are not going to produce results of the required

2. Smolt-to-adult survival. Smolt-to-adult survival estimates are commonly made with **CWTs.** However, it is necessary to kill fish at adult passage facilities in order to recover release group codes from snout-tagged fish making this method inappropriate for estimating pre-spawning adult survival. This would limit snout tag recoveries to those taken on the spawning grounds and from any in-river fisheries. By using tags placed in various body areas pre-spawning fish could be rapidly identified to release group without sacrificing fish.

3. Fry-to-smolt survival. There is a limit in fish size below which **CWTs** begin to loss their effectiveness due to high tag loss, handling stress, and injury from injecting the now relatively large tag into the nasal cartilage (Morrison and Zajac 1987). Standard length **CWTs (1.1 mm)** are recommended for use on fish that are 2.1 g or larger and half length tags (0.5 **mm)** are recommended for marking fish 0.9 g and above (Blankenship 1990).

4. Harvest rate estimates. **CWTs** are the only tag that can address this question on a large geographic scale at this time because of the considerable CWT sampling effort throughout eastern Pacific coastal fisheries by state, provincial, tribal, and federal agencies. No other tag is routinely sampled for on the scale of **CWTs.** Ocean harvest estimates will have greater significance for groups of fish with known high rates of interception, such as fall chinook, and will be less important for other species such as steelhead and spring chinook with much lower interception rates. In-river catch estimates for both the Yakima and Columbia rivers will be important for nearly all species. Recovery rates for experimental and control groups may be so low in many fisheries outside the Columbia River that no clear conclusions can be drawn about differences between release groups.

5. Survival of tagged juveniles past McNary Dam. Coded-wire tagged juveniles are not currently sampled (sacrificed) at McNary Dam. Many tag release groups pass McNary Dam and it will be necessary to kill fish in order to identify Yakima River release group codes.

6. Survival of NxN, NxH, and HxH crosses. Since this question requires a technique that passes a mark across at least one generation, **CWTs** are not appropriate.

7. Trapping efficiency of adult and juvenile trapping and passage facilities. Trapping efficiency can be estimated accurately using **CWTs** (D. Seiler, WDF, pers. **comm.**). However, if multiple releases are made simultaneously to determine differences between treatment and control groups, tagged fish must be sacrificed in order to identify fish to their respective release groups. Body area tags could be used to

identify multiple release groups. No data on minimum size requirements are available yet for this tagging technique. However, at some small fish size body areas may be so close that it is not possible to interrogate the fish and identify each marked body area unambiguously. Minimum fish size for body area tagging is an area deserving further study both in relation to mark recovery and survival.

8. Accuracy of fish identifications. Given that adequate quality controls were implemented during tagging, an accurate count of the number of tagged and untagged fish (including fish having lost tags) will be known. Tag loss is typically much less than 5 percent (Blankenship 1990). All CWT supplemented fish cab be identified by the missing adipose fin and identification of supplemented fish will be essentially 100 percent correct, assuming that the number of naturally occurring missing adipose fins is very low. Typically, hatchery and natural fish lose adipose fins at rates of less than 0.5 and 0.05 percent, respectively (Blankenship 1990). However, clipped adipose fins do not identify fish to specific release and experimental groups. Thus, if there is more than one tag code released, fish will have to be sacrificed in order to identify specific tag codes. Body area tags can provide accurate mark identification information in adults, but do not provide the large number of unique codes the CWT does. Accuracy of juvenile mark identification has not been tested, but should be investigated as this is an area where benign identification methods are needed. Four body areas used in combination will result in 15 unique marks. Increasing the number of body areas to 5 will increase the number of unique combinations or marks to 31.

9. Spawner distribution/stravina. Spatial and temporal distribution of tagged supplemented fish on the spawning grounds can be estimated by recovering tagged fish during spawning ground surveys. However, the accuracy of these estimates will be affected by the number of actual tag recoveries made and the potential bias in sample recoveries mentioned above in the introduction. Estimates made from adults sacrificed at monitoring facilities lower in the river will be limited in spatial resolution and the actual time of spawning will not be known.

10. Species interactions. Juvenile and adult interactions, in terms of spatial/time overlaps with other species or groups (resident/anadromous or experimental/control), can be monitored through CWTs. Overlaps in juvenile distribution in space and time can be estimated by releasing tagged groups and censusing fish using techniques such as electroshocking repeatedly over time within specific study areas (see Leider 1989). As noted above, when multiple tag codes are released, fish must be sacrificed in order to recover the specific tag

alleles, supplemented fish should be nearly identical genetically to natural fish from which the hatchery broodstock was taken and can therefore not be separated using GSI. However, if unique rare alleles are developed and used to mark two groups it then becomes possible to estimate the proportion of marked and unmarked fish. The accuracy with which estimates can be made is dependent on both the rarity of the allele used and the number of alleles used. By using more than one allele, the rarity of each allele can be reduced and the amount of selective pressure necessary to mark the fish reduced. By reducing selective pressure, the likelihood of reducing the fitness of the marked fish through inbreeding is reduced. It is probable that in order to sample sufficient numbers of alleles, tissue from the heart, eye, liver, and muscle must be collected which requires sacrificing fish. In cases where there is no overlap in the allozyme frequencies, individual fish may be identified to a particular group with a high degree of accuracy, although such cases are rare.

9. **Spawner** distribution/straying. As in 8. above, without selective breeding for unique rare alleles, supplemented fish should be nearly identical genetically to natural fish from which the broodstock was taken. Thus, the proportion of supplemented and natural fish cannot be accurately estimated on the spawning groups using GSI methods. The proportion of spawners straying from one identifiable **substock** to another should be estimable. However, unless supplementation fish are uniquley marked the number of supplementation and natural fish straying from a particular **substock** will not be known.

10. **Species interactions.** Provided differences exist in the genetic makeup of the supplemented **species/substock** and the natural population then it should be possible to estimate the rate of interbreeding between groups.

Induced Otolith Bands.

Through the manipulation of water temperature, unique patterns of alternating light and dark bands can be produced on the otoliths of **eyed eggs ,** fry or fingerlings (Volk et al. 1990). These bands persist throughout the life of the fish and temperature changes of as little as two degrees Celsius for 12 hours are sufficient for inducing a detectable mark. The entire egg production of a hatchery facility can be marked with relatively little effort (Volk et al. 1990) making it quite feasible to uniquely mark all fish within any experimental or release group before hatching, eliminating the need to rear the groups separately until they are large enough to tag using other marking techniques. The accuracy of separating natural from hatchery origin fish depends on how **large differences** are between the regular patterns of the induced bands and the irregular natural patterns resulting from random or irregular fluctuations in

natural water temperature. The fewer the number of groups, the simpler the pattern for each group can be and the less likely it is that confusion between groups will occur. The number of groups which can be uniquely marked and accurately identified is not known at this time, but should be quite large (easily more than 100). To date the technique has been used to identify juvenile and adult experimental and control groups of chinook, coho, and chum returning to particular hatchery facilities (Volk et al. 1990) and to address mixed-stock interception problems in pink and sockeye salmon (Crandall et al. 1990). To date otolith banding patterns have not been used to separate naturally rearing from hatchery populations or to characterize naturally rearing substocks of fish, although it is anticipated that separating naturally rearing and hatchery populations will not be a significant problem (E. Volk, WDF, pers. comm.).

The biggest disadvantage to otolith marking is that mark recovery requires sacrificing all fish within a sample, both marked and unmarked, in order to identify marked fish and recover release group codes. Since there is no external mark, such as an adipose fin-clip, marked and unmarked fish cannot be sorted out. However, otolith bands can be applied to very small fish without the potential negative effects associated with other marking techniques such as olfactory nerve damage, tag loss, altered behavior and stress. In addition, otolith marks can be applied to very large numbers of fish in the eyed-egg stage prior to hatching at very low cost.

In any study using otolith banding patterns, the total number of otolith samples that must be processed in order to recover 35 fish per replicate group will depend on the proportion of the mixed-group population that is otolith marked. If marked fish from all release groups represent only 1 percent of the fish being sampled then a large number of otoliths must be processed in order to recover 35 marked fish per release group. However, if the mixed-group being sampled is made up 95 percent marked fish, nearly every fish has a mark and the total number of otoliths to be processed in order to recover the same number of marks will be significantly lower.

Capital costs for water chillers, retrofitting plumbing, and an pump system at a typical hatchery capable of marking 400,000 eggs resulting in 200,000 marked smolts (assuming a very conservative survival from egg to smolt of 50 percent) is less than $10,000 plus the cost of electrical service modification which can range form $1,000 to $9,000 (E. Volk, WDF, pers. comm., 1989). These are one time capital costs, while the cost of operating the chiller system over a typical marking cycle (about 4-6 weeks) is very low. Chillers, etc. are not needed in facilities which have natural sources of water with 2° C or more differences in temperature, i.e. well and creek sources. In relatively simple situations requiring

only visual identification of characteristic banding patterns, otolith processing and analysis should require about one man-month per 1,000 fish (E. Volk, WDF, pers. comm., 1989).

1. Release-to-smolt and smolt-to-smelt survival. Since fish must be sacrificed for mark recovery this technique can only be used in situations where killing fish is not a serious consideration. Marked otoliths can be used to estimate these survival rates, but if the marked groups make up a very small proportion of the total population, it will require killing a relatively large number of fish to recover sufficient numbers of marked fish. When the proportion of marked fish is large relative to the unmarked portion, tag recovery rates will be much higher and the total number of fish sacrificed will be lower.

2. Smolt-to-adult survival. Due to the fact that fish must be killed or recovered dead to extract mark information, otolith banding is best suited to estimating adult survival to the spawning grounds from carcass recoveries. Monitoring adults for otolith marks at adult passage facilities will probably require killing an unacceptably large number of both natural and hatchery fish prior to spawning. In limited cases where sacrificing adult fish is not considered a significant problem, marking with otolith banding patterns could be quite useful.

3. Fry-to-smelt survival. Otolith bands could be a particularly good method for evaluating this question since fish can be marked as eyed eggs with no ill effects. This means even unfed fry outplants could be marked. However, the fact that fish must be sacrificed to recover the mark limits the technique to situations where fish can be sacrificed without creating significant risk to the populations being sampled and where the marked fish make up a large proportion of the population being sampled.

4. Harvest rate estimates. This technique could be used to address this question. However, an otolith sampling program would need to be initiated. Otoliths from known unmarked and marked hatchery and natural populations could be analyzed as a blind sample and the accuracy of the technique estimated.

5. Survival of taaaed iuveniles past McNary Dam. This is not an appropriate technique at this time to address this question because such a low proportion of fish would be otolith marked that an unacceptably high proportion of fish would need to be sacrificed in order to recovery a sufficient number of marks. Any statistical analysis would also need standards representing all known groups passing NcNary Dam.

6. Survival of NxN, NxH, and HxH crosses. This question typically requires a mark that is passed across at least one generation. However, in a limited experimental setting known numbers of otolith marked eyed eggs of known parental crosses could be placed into a controlled stream section and allowed to incubate naturally. The emerging juveniles could then be sampled at various early life history stages and the number of progeny from each cross identified. Survival, growth, emergence timing, incubation rates, and possibly post-emergence distribution patterns and behavior of each cross could then be estimated and the relative performance of each cross measured. This experiment requires controlling the stream environment and would be best performed in an artificial stream channel.

7. Trapping efficiency of adult and juvenile trapping and passage facilities. This question could be addressed with otolith marks when small fish, which are below acceptable marking size using other techniques, must be marked. However, both marked and unmarked fish must be sacrificed for mark recovery and the cost in terms of lost fish production will probably be to high in most cases.

8. Accuracy of supplemented fish identification. Experimental hatchery release groups can be identified with a high degree of accuracy (Volk et al. 1990). The technique has not been applied to studies which include natural populations, yet. The accuracy with which natural and hatchery origin fish can be identified must still be assessed. Due to the regularity and predictability of the induced otolith marks compared to the less controlled natural populations, it is anticipated that the accuracy of identifying hatchery and natural groups will be high. Accuracy of natural group identification is unknown.

9. Spawner distribution/straying. Otoliths collected from spawning ground recoveries can be used to estimate the spatial and temporal distribution of marked fish post-spawning. However, the accuracy of estimates will be affected by the number of marks recovered and the bias in sample recoveries mentioned above in the introduction. The number of fish which must be sacrificed for mark recovery at adult passage facilities'will probably be unacceptably high and estimates made from adult passage mark recoveries will be limited in spatial resolution.

10. Species interactions. Otoliths are probably of limited use in addressing interaction questions since releasing mark fish for future recovery and benign mark recovery is typically required.

Elemental Marking (EM).

Recent advances in mass spectrometry techniques, such as inductively coupled plasma mass spectrometry (ICPMS), now make it possible to measure the concentrations of many elements simultaneously at the ppm to ppb level while requiring relatively small amounts of material for analysis (<1 mg). This advancement in analytical technology has revived interest in EM as a method for marking groups of fish (Coutant 1990). Research has focused on measuring the concentration of elements in scales and other calcified hard parts. Elemental marking involves increasing the concentration of an element or multiple elements to levels significantly greater than natural background concentrations. These elements often replace calcium in the bony structures. The elements are permanently incorporated into a narrow concentrated band within the matrix of the hard parts and do not degrade significantly over time. Typically, less than 1 mg (l-3 non-regenerated adult scales) of material is needed for elemental analysis. By removing scales from live fish and measuring the concentration of elements of interest, marked and unmarked fish have been identified with very close to 100 percent accuracy (Behrens Yamada and Mulligan 1990; Bob Brown, Elemental Research, pers. comm., 1989). Thus, mark recovery does not require sacrificing fish.

Care must be taken to evaluate this marking techniques to insure no negative effects on growth and survival are associated with the elements used in the marking process. A number of elements have successfully been demonstrated as useful for fish marking purposes including: samarium, lanthanum, and cerium (Brown 1990), strontium, rubidium, and manganese (Behrens Yamada and Mulligan 1990) and europium (C. Coutant, Oakridge National Lab, pers. comm.). Two groups of coho, one marked with CWTs only and the other strontium and CWT.-marked, were released by Pacific Biological Station personnel and recovered as adult hatchery returns 18 months later. No difference was found in smolt-to-adult survival of the two release groups (T. Mulligan, PBS, pers. comm.).

Marking costs are unknown at this time because the most effective elements and form of introducing the mark; feeding or immersion, has not yet been determined. The elements currently being tested such as strontium, lanthanum, cerium and a number of the other rare earth elements are relatively inexpensive. For example, using strontium chloride in feed it cost $3.40 for the materials used to mark 200 juvenile sockeye (Behrens Yamada and Mulligan 1990). Using this estimate it would require $3,400 of materials to mark 200,000 fish. Actual cost would probably be much lower when elements are purchased in bulk. The cost of any associated equipment for effluent treatment cannot be made at this time.

Mark recovery costs are also uncertain at this time, and will depend to a large degree on the analytical technique used, the

total number of samples to be processed, and the number and kind of elements which must be measured per sample. Current ICPMS costs are $30 per element per fish for relatively small lots of samples, although the cost per sample may be closer to $10 per element per fish when large numbers of samples are processed (B. Brown, Elemental Research, pers. comm.). Actual ICPMS analysis of a sample takes only about two minutes.

Work still needs to be done on a number of significant concerns, such as: the most efficient delivery system for EM (immersion or feed), the toxicity and recommended dosage for elements, FDA approval allowing release of elementally marked fish, and environmental (EPA) concerns about effluent from either feed or immersion. Other issues of concern include background levels of elements in hatchery reared and natural populations, feasibility of using multiple-element marks so that combinations of elements can be used to uniquely identify replicate releases or multiple release groups, and improving microanalytical techniques so that spatial information (where on the calcified tissue the mark occurs) can be obtained. These last two concerns are important, since spatial resolution and multiple-element marking provide the opportunity to create a much greater number of unique marks. Microanalytical techniques that give spatial resolution will also minimize dilution problems and increase sensitivity (see 8. below). FDA approval for each marking compound must be obtained or the technique, no matter how successful at marking fish, will be impossible to apply.

Progeny of anadromous and non-anadromous steelhead/rainbow trout have been identified using microanalysis of strontium concentrations in otolith nuclei (Kalish 1990). Progeny of anadromous steelhead have higher levels of strontium in their otolith nuclei than progeny of rainbow trout because the eggs of anadromous steelhead absorbed strontium while the female is immersed in saltwater, which contains significantly higher levels of strontium than freshwater. This technique could be applied at this time to determine whether adult steelhead and rainbow trout were progeny of anadromous or nonanadromous females by measuring the strontium content of their otolith nuclei. Fish with elevated levels of strontium are progeny of anadromous females. Adult return could be otolith sampled and the relative contribution of each life history type (anadromous dam/anadromous progeny, anadromous dam/nonanadromous progeny, etc.) could then be estimated.

Another intriguing potential application of EM is to intentionally mark progeny by injecting gravid females with a solution of a marking element causing the eggs to absorb the marking element. After fertilization, the element should be incorporated into the developing otoliths of the fish. In this way, the otoliths of the female's progeny are marked with a concentrated band of the introduced element. Females of hatchery origin could be marked with one element and natural females with another. Females could be confined in a controlled stream section and allowed to mate with

males of either hatchery or natural origin. The progeny of these matings could be sampled and maternal origin identified. If the size, age, and number of fish in each group of marked females is **equal** we would expect the number of resulting fry or smolts to be equal across groups, as well. By measuring the resulting production we have a measure of reproductive success of each mating type under essentially natural conditions. Work should begin immediately to develop this method of marking and microanalytical methods for otolith analysis should be developed.

As with otolith banding studies, the total number of calcified tissue samples that must be processed in order to recover 35 elementally marked fish per replicate group will depend on the proportion of the mixed-group population that is elementally marked. If marked fish from all release groups represent only **1** percent of the fish being sampled then a large number of tissue samples must be processed in order to recover 35 marked fish per release group.

The functional equivalent of the adipose fin clip which allows **quick** visual identification of CWT-marked fish is currently lacking. WDF is now in the process of developing a method of marking scales with a fluorescent mark which could be used as a generic mark for all EM and otolith marked fish. Scales would be removed from fish and passed under an epi-fluorescent microscope. Fluorescently marked fish would then be identified (all release groups are fluorescently marked). In the case of EM mark recovery, only scales from fluorescently marked fish would be analyzed for elemental marks which would identify the particular release group code.

In the following ten discussion points it is assumed that the major questions regarding EM have been satisfactorily answered such as acquiring FDA approval, establishing effluent treatment protocols, and determining the most efficient mark application methodology.

1. Release-to-smolt and smolt-to-smelt survival. EM could be used to estimate these survival rates. An entire group of fish could be marked before release via diet ***or*** immersion., The number of fish passing various monitoring facilities can then be estimated by collecting scales from live fish, analyzing the scale sample for elemental composition, and identifying the marked fish by their elevated levels of specific elements. Sacrificing fish will not be necessary.

2. Smolt-to-adult survival. Live adult fish could be scale sampled at passage facilities and the marked fish identified by the significantly higher levels of specific elements. From this information the total number of returns for each marked group can be estimated and survival rates for each group estimated without sacrificing fish.

3. Fry-to-smolt survival. Fry begin forming scales at about three-four centimeters. Thus unfed fry would probably not have scales developed at release. EM looks to be a promising low stress way to mark small fish with scales which may not be efficiently or effectively marked with other techniques. WDF has successfully marked emergent sockeye and chum fry otoliths and vertebrae using a stable isotope of strontium.

4. Harvest rate estimates. Estimates of harvest rates outside of the **Columbia/Yakima** river systems would require a great deal of sampling effort and require processing a very large number of samples. Harvest rates of in-river fisheries are possible if sampling programs were implemented and **the** populations contributing to the fisheries are baseline sampled to determine the background levels of the elements used in marking.

5. Survival of **tagged** juveniles **past McNary** Dam. It could be possible to estimate smolt survival to McNary Dam using EM. Baseline information on the other populations passing McNary Dam would be needed in order to be certain the population of interest is uniquely marked. In addition, EM sampling juveniles would have to be included in the screening protocol for juveniles passing through the dam. If the marked population makes up a small proportion of the mixture then large numbers of marked and unmarked fish will need to be processed unless a fluorescent mark on the scales is used to sort put marked fish.

6. Survival of **NxN, NxH,** and **HxH** crosses. This question requires a mark that can be passed across at least one generation. **It** may be possible to mark the otoliths of progeny of gravid females by elevating the level of one or more trace elements in the females body cavity through injection of a marking solution prior to spawning. The eggs would then absorb the trace element and incorporate it into their developing otoliths after fertilization. This method would be done on an experimental basis rather than production scale and would require sacrificing juvenile fish in order to collect otolith samples. A section of a tributary or artificial stream channel would need to be segregated into four equal sections of comparable spawning quality. Into each section equal numbers of uniquely marked hatchery or natural origin females of comparable age and length would be released. Into each section either hatchery or natural origin males would be released and the adults allowed to spawn. In this way the four possible matings of hatchery and natural males and females are made: **H** ♂ x **H** ♀, **H** ♂ x **N** ♀, N ♂ x N ♀, and N ♂ x **H** ♀. The fry from each section would then be sampled at emergence or after a longer period of development. Since the females in each of the four sections are uniquely marked, separating the stream sections after spawning will not be necessary and progeny from

each mating (i.e. H ♂ x Ii ♀) will be identifiable. Otoliths of surviving juveniles can then be analyzed to determine the parental origin of each fish. This data can then be used to estimate and compare the relative survival or reproductive success of each cross.

7. Trapping efficiency of adult and iuvenile trapping and passage facilities. Trapping efficiency could be estimated using EM. Trapping efficiency can often be related to the size of fish and EM may be one method for evaluating trapping rates of very small fish which cannot be tagged easily with other techniques. In addition, it would not be necessary to sacrifice fish for sample collection. For larger juvenile fish other less analytically complicated techniques would be recommended.

8. Accuracy of supplemented fish identification. Current research with rare earth elements and strontium have resulted in very high classification accuracies (>95 percent) of juvenile experimental and control groups (Behrens Yamada and Mulligan 1990; T. Mulligan, pers. comm., 1989; B. Brown, pers. comm., 1989). Adult identification can be complicated by a dilution effect due to the growth of the fish's hard parts not to loss of the marking material. For elements that are rare throughout the fish's rearing environment and in concentrations above detection limits, the dilution effect may have little or no significant effect. However, for elements which are not rare, such as strontium (present in sea water at a relatively high concentration), the additional material taken up over the ocean rearing phase can dilute and obscure significant difference between experimental and control groups (Behrens Yamada and Mulligan 1990). By excising the freshwater portion of the scale using a "cookie cutter" technique and analyzing only the excised portion, this problem can be overcome. Other analytical techniques are being investigated as ways to overcome the dilution effect as well, such as vaporization furnaces and laser ablation.

9. Spawner distribution/straying. EM could be used to estimate the number of fish passing through an adult passage system by collecting and analyzing scale samples without sacrificing fish. Estimates of migration timing and spatial distribution can be made to a level of resolution determined by the monitoring facilities. Actual time of spawning will be unknown, however. Scale samples collected from spawning ground recoveries can be used, as well. However, the data may be affected by biases in sample recoveries mentioned above in the introduction.

10. Species interactions. Juvenile fish could be identified to a particular mark group through EM analysis of scale samples. It should be possible to identify a fish to its respective

release group each time it is recovered and measure response variables such as spatial and temporal overlaps in rearing and monitor movements of release groups. Scale samples should be spread out randomly over a large portion of the fish's body. This should be done in order to avoid taking scales from exactly the same place twice since new scales will be regenerated to replace those lost in any previous collection and all scales regenerated after elemental marking will lack the mark.

Visible **Implant (V.I.) Tags.**

V.I. tags are similar in concept and use to the CWT (Haw et al. 1990). Tags are small (approximately 0.6 x 1.5 x 0.13 mm), have a relatively low impact on fish behavior and survival compared to external tags and fin mutilations. In salmonids, the V.I. tag is generally injected into the clear adipose eyelid tissue just posterior to the eye. The important difference between **CWTs** and V.I. tags is that V.I. tags can be visually decoded without sacrificing fish. Individual fish can be tagged with unique codes, as well. As juvenile and adult fish are sampled at trapping sites, fish would be examined for the presence of a V.I. tag, the tag code visually read if present, and the fish promptly released to continue its migration. Thus, fish can be sampled multiple times and individuals tracked temporally and spatially to determine migration/distribution trends, growth rates and survival. V.I. tag codes are alphanumeric and can be identified with a particular experimental group or individual fish, if need be. As the current state of the tag now stands, salmonids less than 150 mm are not recommended for tagging (L. Blankenship, WDF, pers. **comm.**, 1989). Steelhead and spring chinook smolts would appear to be the most likely species on which to apply this type of tag. The technology has not been used to mass mark hundreds of thousands of fish yet, although 8,000 V.I. tagged steelhead smolts were released from one Washington state hatchery in 1991.

Current cost-per-tag estimates are about **$20-30/100** tags depending on the quantity ordered. However, V.I. tag data collection and decoding costs should be several orders of magnitude less than for **CWTs,** recovery information should be available almost immediately, and sacrificing fish is not necessary. Specialized lab equipment and trained personnel for tag decoding, as well as dedicated facilities for handling and storing samples, would not be necessary.

Two other newly developed benignly recoverable marking methods similar in concept to the V.I. tag are **CWTs** and fluorescent materials injected either into the adipose fin, between ventral fin rays or into adipose eyelid tissue. These techniques are currently being developed and tested on juvenile fish by Northwest Marine Technology (NET). Costs are expected to be an order of magnitude

cheaper than V.I. tagging and applicable to fish about 100 mm in length (L. Blankenship, WDF, pers. comm.). Using different colors and mark locations more than 15 different release codes are possible. If individual fish must be identified upon return then individually numbered CWTs can be injected into the adipose fin which can be excised on return (Haw 1991). Tagged fish would have their adipose fins removed with the tag in place. A numbered jaw tag can then be placed on the fish and upon subsequent recovery the fish can be identified to its respective CWT code. On the spawning grounds jaw tagged carcasses with missing adipose' fins could either have a snout CWT still in place or have had their adipose fin CWT removed. Snout CWTs should be recovered from carcasses so a means to identify snout CWT fish as carcasses would be useful. This could be accomplished by jaw tagging fish ad-clipped as juveniles with a unique jaw tag code which would immediately identify these fish on the spawning grounds. It will then be clear which jaw tagged fish have CWTs in their snouts. Fish with missing adipose fins and no jaw tag are known to have been ad-clipped as juveniles, assuming jaw tag loss is insignificant. Any of the response variables which can be addressed with V.I. tags can probably be addressed equally well with these tagging methods and, in cases where fish less than 150 mm must be tagged, one of these other techniques may be a better choice.

1. Release to smolt and smolt-to-smolt survival. The V.I. tag should give reasonable results to questions dealing with smolt survival provided the size at tagging is large enough and the number of fish to be marked is not prohibitively large. The initial investment in tagging fish and subsequent dollar loss due to mortalities before sampling, such as occur in spring chinook, will be greater that for CWTs but at least one order of magnitude lower than for PIT tags. V.I. tag data recovery will be labor intensive due to fish handling but relatively cheap and available immediately without sacrificing fish. Individual fish can be identified, just as with PIT tags.

2. Smolt to adult survival. V.I. tags are an appropriate marking technology for addressing this question provided fish are large enough prior to release. The study and analysis would be very similar to a CWT study, except that fish would not be sacrificed to decode the tag. Also, if fish are individually marked, age and growth can be related back to release size for each fish and the relationship of release size to the proportion of jacks returning and age at return can be investigated. Tag shedding may be a significant problem in carcass recoveries as fish decompose although this has not been tested.

3. Fry to smolt survival. Fry are below the minimum size (150 mm) which can be tagged using V.I. tags. Therefore, this question cannot be addressed using current V.I. tag

technology. Other benignly recoverable techniques mentioned above can be used on fish down to 100 mm.

4. Harvest rate estimates. Although there is currently no sampling plan for recovering V.I. tags from inside Columbia River fisheries, a concerted effort to collect V.I. tag data might be possible without a large investment in labor or money by simply alerting current samplers to the tags presence. Therefore, this question could be addressed using V.I. tags, particularly within the Yakima River.

5. Survival of tauaed iuveniles past McNary Dam. Although sampling for V.I. tags is not currently done at McNary, it could be done while checking juvenile fish currently being screened for freeze brands at McNary Dam. Data on each experimental release group could be then be collected. If it were necessary to monitor the migration of individual fish for migration timing trends and survival studies, this could be done by tagging fish with individually numbered V.I. tags.

6. Survival of NxN, NxH, and HxH crosses. Since this question requires a techniques that passes a mark across at least one generation, V.I. tags are not appropriate.

7. Trapping efficiency of adult and iuvenile trapping and wassaue facilities. V.I. tags could be used to address trapping efficiency questions in the same manner that the CWT. would be used, accept that sacrificing fish would not be necessary. This would allow the release of replicate groups or multiple experimental groups and not require killing fish to get recovery information on each group. In addition, if fish are individually marked, size at release can be examined in much greater detail and its effect on trapping efficiency, migration timing and survival studied.

8. Accuracy of suwolemented fish identification. As with CWTs, once the V.I. tag code has been recovered visually, fish should be identified with 100 percent accuracy to the appropriate experimental/release group, provided tag loss is minimal. If all supplemented fish are V.I. tagged, returning adults can be checked, identified as supplemented or not, identified to a specific tag group or fish, and finally released unharmed. Decoding of the tag is immediate.

9. Swawner distribution/straying. Spawning ground surveys can be made and all recovered fish checked for V.I. tags. Tag shedding by post-spawning adults is unknown at this time, but is probably high for carcasses as they decompose.

10. Species interactions. Juvenile and adult interactions in terms of spatial/time overlaps with other species or groups (resident/anadromous or experimental/control) can be monitored

using V.I. tags. Overlaps in juvenile distribution in space/time can be estimated by releasing tagged groups and censusing fish using techniques such as electroshocking over time in various study areas (see Leider 1989). Since fish are not sacrificed, multiple recoveries are possible and estimate on residence time and movements are possible for individual tag groups or fish. Recoveries of V.I. tagged adult fish can be used to estimate the degree of spatial/temporal overlap in spawning distribution between different species, stocks or experimental/control groups, as well as, adult survival rates subject to the relatively low recovery rate and bias problems described in the introduction.

Passive Inteurated Transponder (PIT) tags.

PIT tags possess the advantages of the V.I. tag with the additional benefit of automatic data logging by remote sensors in juvenile fish passage facilities. Thus, handling fish for data collection is eliminated and approximately 95% or more of the fish passing through the monitoring device are interrogated. In addition, each individual fish can be marked uniquely. According to' Prentice et al. (1990)I there are no measurable negative affects on fish growth, survival, respiration rate, tail-beat frequency or stamina due to PIT tagging fish as small as 55 mm (1.6 g). It is difficult to understand how a tag that is 22 percent of a fish's body length and perhaps 50 percent of the body cavity length can have no significant effects on growth, behavior and survival of such small fish during the critical first few days after release. The PIT tag's effects on survival, behavior, and growth of actual releases of small fish deserves more rigorous study in order to identify a minimum recommended size for tagging. Many researchers feel comfortable tagging fish 80 mm and larger (D. Maynard, NMFS, pers. comm.).

The benefits of automatic data collection come at a cost. PIT tags are currently priced at about $3-4 per tag depending on the quantity ordered. In addition, capital construction costs associated with installation of juvenile monitoring sensors are high, sensor arrays are expensive ($60,000 per set of three coils and more than one set is generally needed per monitoring station), and maintaining the computerized data collection system is an additional continuing cost factor.

PIT tag monitoring of adult returns at mainstem Columbia River dams using current technology has been determined to be unfeasible a", this time due to problems associated with adult interrogation systems (see DeHart 1991). These problems include FCC licensing requirements, radio frequency interference from other on site electrical systems (e.g., rheostats, electric motors), and shielding fish and personnel from high frequency radio waves. In addition, tag shedding in Skagit River coho has recently been

estimated to be about 60 percent in females and as high as 20 percent in males (D. Maynard, NMFS, pers. comm.). Tag shedding has been documented informally in other Pacific salmon species at or near spawning as well, but has not been as high as in coho and has not been detected in males at this time. Significant tag shedding of a larger tag (17 mm x 7.5 mm diameter) has been documented in Atlantic salmon after 4 months (Moore et al. 1990). Tag loss by maturing adults puts into question any estimates based on either spawning ground recovery of PIT tags or monitoring adults in the lower river. Until it is known at what period in the fish's life history tag shedding becomes a serious problem, pre-spawning in-river data will be suspect.

A plastic jaw tag with an implanted PIT tag has been tested in the Columbia River and could be a useful tool for monitoring adult in-river movements. The usefulness of this tool is dependent on a satisfactory adult monitoring system being designed. NMFS's most recent plan for adult monitoring is to put development of the current high frequency 400 KHz technology on hold and wait for the newly developing lower frequency 125-132 KHz technology to emerge and provide new options that will lead to more feasible adult monitoring systems. The lower frequency technology allows 2-3 times the interrogation range, probably eliminates the need for FCC licensing, reduces radio frequency interference, and may be less expensive overall. It will be a minimum of one year before such developments take place according to E. Prentice (NMFS).

Monitoring PIT tagged juvenile fish at dams along the Columbia River, including McNary, would allow estimates of the number of marked smolts from each group passing and the duration of migration. If PIT tagged fish are diverted and measured, growth after release for each fish within experimental and control groups can be estimated, as well. However, size biased trapping efficiency, which is not equal at each dam (Giorgi et al. 1988, Giorgi 1990) and may not be equal at each monitoring site, must be understood before accurate estimates of passage can be made. This problem is not unique to PIT tags and exists for any marking technique which uses recoveries at multiple dam/monitoring sites.

A NMFS proposal has been made to develop a new generation of PIT tags using acoustic frequencies rather than the higher frequency range of the current PIT tags (Prentice 1991). This tag would boost the detection range to about 40 feet allowing interrogation of fish in situ in many instances. The tag would be compatible with existing PIT tagging infrastructure such as tag injectors, data handling and tag size, although remote detection systems would have to be replaced. Adult tag shedding problems would still exist however, since no change in tag encapsulation materials is anticipated. No working prototype of the tag has been constructed yet and the technology remains on the drawing board at this time.

1. Release to smolt and smolt to smolt survival. PIT tags can effectively address this question. It is anticipated that in many situations survival will be low between tag release and recovery necessitating the release of a large number of tags no matter what tag/mark is used. However, the high cost of PIT tag costs will be offset to some degree by the need to release fewer tags since automated tag recovery typically allows more fish to be sampled and consequently higher numbers of recoveries. This benefit will be greatest when the volume of fish to be handled is high. When fish volumes are low and can be interrogated just as easily by hand or other method, PIT tags lose much of their advantage relative to cost. Handling of fish can be reduced by a ratio of **33:1** in main stem Columbia River **CWT** and brand monitoring (Prentice et al. 1986).

2. Smolt to adult survival. Due to the high rate of tag loss demonstrated in spawning **coho** salmon spawning ground recovery of PIT tags is not recommended. The time period tag shedding occurs must be identified in order for PIT tags to be used as a reliable method for estimating adult survival to lower river cites. Tags may be being shed as the fish are maturing and gametes begin to loosen up, thus allowing tags to more easily migrate out of the body cavity. If this is true, then tag loss may occur well before spawning occurs and mark recovery data on fish collected at lower river facilities may be biased by tag loss, as well. Survival rates are such that entire release groups must be tagged in order to recover a minimum number of tags for statistical analysis.

3. Frv to smolt survival. It is not recommended that fish as small as 55 mm (1.6 g) be PIT tagged and released as recommended by Prentice et al. (1990). Until a rigorous study is performed to look at the PIT tag's effects on actual releases of fish of varying size under operational conditions, the minimum recommended size for tagging should be about 80 **mm.**

4. Harvest rate estimates. Unprocessed adults carcasses (viscera intact) can be interrogated using a hand held tag detector making mark recovery from fishery samples possible. However, the time period when adult tag shedding becomes significant has not been identified and may begin as early as entrance into freshwater.

5. Survival of taaaed juveniles wast **McNary** Dam. **McNary** Dam currently has sensors installed that will detect PIT tags as they pass downstream making PIT tags an excellent technique for monitoring passage of juveniles through dam sites. Adult monitoring systems have not been developed or installed.

6. Survival of NxN, NxH, and HxH crosses. Since this question requires a technique that passes a mark' across at least one generation PIT tags are not applicable.

7. Trapping efficiency of adult and iuvenile trapping and passage facilities. PIT tags are an excellent way to monitor the passage of smolts to determine trapping efficiency. Yearling fish should be large enough to be tagged. Smaller sub-yearling fish may not be large enough to tag without affecting migration behavior and survival, however. Adult trapping efficiency could be estimated if the tags were attached to the jaw with a plastic cinching jaw tag. However, a viable method of interrogating adults would need to be developed and installed at the monitoring facility. Although it may be possible to fit small scale adult passage facilities such as at Prosser with 400 KHz adult PIT tag monitoring equipment it would be prudent to wait for the development of the low frequency hardware and not have to replace obsolete 400 KHz equipment after possibly one or two years.

8. Accuracy of suwwlemented fish identification. Once PIT tags have been implanted, tagged juveniles should be identified with 99 percent accuracy (Prentice et al. 1990). In addition, large numbers of juvenile fish can be automatically interrogated (20,000/hour at a ratio of 1:4 PIT tagged to untagged fish) using remote sensors at 93 percent detection efficiency or higher. Adult interrogation rates for coho salmon have been estimated at 360 fish per hour (Des Maynard, NMFS, pers. comm.). However, when juvenile and adult fish volitionally move through a monitoring system they can "park" in the tag interrogator causing the tag to be read many thousands of times. This becomes a problem when the orientation of the fish changes and radio frequency interference occurs causing many tag reading errors to occur. These errors must be identified and corrected. Adult tag shedding is a major problem making PIT tags inappropriate for maturing adult survival experiments at this time. PIT tags embedded into jaw tags and placed on adults should be accurately identified once the adult monitoring technology is developed.

9. Swawner distribution/stravina. Adults marked as juveniles shed PIT tags making this marking method inappropriate for this question. When used in concert with a benignly recoverable mark placed on juvenile fish, adults jaw tagged with PIT tags could be used to estimate straying of supplemented fish, time of entrance, rates of movement between monitoring points, and fall back rates by release group provided adult monitoring equipment were developed and installed.

10. Species interactions. Juvenile interactions in terms of spatial/time overlaps with other species or groups (resident/anadromous or experimental/control) could be monitored using PIT tags and would not require sacrificing fish for tag recovery. Overlaps in juvenile distributions in space/time and survival can be estimated by releasing tagged groups and censusing fish using techniques described by Leider (1989). Fish can be interrogated with a handheld PIT tag detector for code recovery and released unharmed. If fish are tagged with individual codes, movements of individual fish could be traced over time. Limits on the minimum size of fish which can be tagged without significant behavioral and/or survival effects have not been established yet. Recoveries of PIT tagged adult fish cannot be made with confidence at this time due to tag shedding by mature fish prior to spawning.

Individually Numbered Jaw Taus

Jaw tags have been used extensively in fisheries studies at WDF (D. Seiler, WDF, pers. comm). Jaw tags could be used in the YKFP to measure adult response variables such as migration rates between adult monitoring facilities within the Yakima River, run timing of natural substocks and hatchery release groups, population estimates (mark-recovery), fall back rates, and assessing biases in mark recovery and demographic data collected from spawning ground recoveries. Such information could be stratified by length, sex, and age if sufficient numbers of fish were recovered. As fish are passed upstream at an adult monitoring facility, fish already tagged or marked to identify release group would be identified, length/sex/age data collected, and an individually numbered jaw tag attached. Naturally rearing returns could also be jaw tagged in order to monitor their movements and numbers. However, natural straying rates could not be estimated unless the origin of the fish can be identified.

Jaw tags would be particularly useful in conjunction with removable tags, e.g. CWT in the adipose fin. Once a removable tag is excised, the fish can no longer be identified to its release group. However, fish can be jaw tagged at the time the removable tag is collected and can then be identified to release group upon subsequent recapture.

The V.I. tag may be able to serve the same purpose as the jaw tag, although it has not been tested or utilized in such a manner at this time. V.I. tag retention and visibility in adults is not known and tag shedding may occur at high rates in post-spawning fish and decomposing carcasses. A jaw tag is more firmly attached and will probably have a longer retention time for spawning ground recoveries.

WDF has used jaw tags to estimate coho population sizes within the Chehalis River system (D. Seiler, WDF, pers. comm.), as has the Skagit System Cooperative (Hayman 1990). Using an opercle punch as a second tag to assess jaw tag loss, WDF found that over two years of jaw tagging coho salmon at Bingham Creek no lost jaw tags were detected in spawning ground recoveries (S. Neuhauser, WDF, pers. comm.). Jaw tag loss and loss of the identification numbers has been a problem when plastic encased "hog ring" tags were used (Hay-man 1990).

1. Release-to-smolt and smolt-to-smolt survival. Not appropriate for this question.

2. Smolt-to-adult survival. Not appropriate for this question.

3. Frv-to-smolt survival. Not appropriate for this question.

4. Harvest rate estimates. Not appropriate for this question accept for fisheries occurring above the lowest adult monitoring/jaw tagging site.

5. Survival of taaaed iuveniles wast McNary Dam. Not appropriate for this question.

6. Survival of NxN, NxH, and HxH crosses. Not appropriate for this question.

7. Trawwina efficiencv of adult and juvenile trapping and passage facilities. Adult passage facilities could be tested using jaw tags. Juvenile fish are too small to be jaw tagged. If PIT jaw tags are used an adult PIT tag monitoring system must be in place.

8. Accuracy of fish identification. Accuracy of tag identification should be very high. Tag loss and loss of identification numbers is not a problem when using aluminum bird band tags. Tag loss rates for PIT jaw tags (electrical cinch straps) is unknown at this time.

9. Swawner distribution and stravina. Spawner distribution (temporal and spatial) and population estimation are the primary areas where jaw tags can contribute. Run timing trends from mark/recapture data, temporal movement patterns between monitoring sites, and straying of known origin fish can be estimated. There are a number of methods available for making estimates of population size based on mark/recapture data.

10. Species interactions. Jaw tags are not likely to be a major tool in measuring species interactions, although information on intra-specific interactions between supplemented and natural adult returns should be useful.

Brands

Brands are not detectable over long periods (2-5 years) with a high degree of reliability (Coombs et al. 1990). Short term use may have applications when detection periods are short (weeks) and other techniques are not available or usable. Brands are typically created by scaring the surface of a fish using metal brands cooled to very low temperatures. This allows the fish to be visually identified to its release group although brands often are not visible for the first few days after application. Problems can occur when fish are stressed during marking because of factors such as poor handling or prior disease infection causing increased mortality. However, almost all marking techniques are subject to these problems to some degree. The effects of the branding process can be variable, depending on the methods used and the experience of the individuals in marking fish. Prentice et al. (1990) found that freeze brands were recovered at significantly lower rates than PIT tags in some cases. The reasons for the lower recovery rate were not identified but may have been due to either the marking technique, brand sampling technique, or the method used to estimate (expand) brand recoveries at the dam facility.

Scale Pattern Analysis (SPA)

Fish have a record of their growth history recorded in the patterns of circuli on their scales. Fish which have different growth histories, due to different rearing environments and/or genetic makeups, will have different patterns of circuli on their scales. Based on differences in scale patterns between known origin groups of fish, mixtures of scales made up of the baseline groups can be analyzed and the proportion of each group estimated. Statistical methods used include linear discriminant analysis (LDA) and maximum likelihood estimation (MLE). It is not necessary that size at release differ significantly between groups, as long as growth rates differ during some portion of their lives. As a general rule, the more groups there are to discriminant between, the lower the accuracy of estimates will be. All potential groups contributing to a statistical stratum must be represented by baseline information in the model. Fish from unrepresented groups which occur in a mixture sample will classify to the group they most closely resemble.

Scale sample processing, measurement, and analysis of 8,000 samples requires approximately one man-month. Typically, sampling of baseline groups must be done on a yearly basis due to inter-brood year differences in scale patterns. Baseline samples are typically between 100-200 samples per group and each statistical stratum (e.g. fishery week) should be made up of at least 100 fish. Fewer fish may be necessary when classification accuracy is high (>90 percent for all groups). Discriminating between release groups using SPA is not likely to be successful since it is unlikely

differences in growth between treatment, replicate, and control groups will be sufficiently large to create significant differences in scale patterns between release groups. Natural **versus** supplemented group separations will have the highest likelihood of success. Natural Yakima spring chinook **substock** discrimination has not proven to be successful to date (see Chinook **Substock** Identification section). No discussion of the ten points follows for SPA.

Parasites

Parasites have been used to differentiate between stocks of salmonids, particularly in sockeye salmon (Moles et al. 1990, Konovalov 1971). The presence or absence of a species of parasite is determined from baseline samples of tissue typically removed from the brain or internal organs. The fish can then be identified to the stock or group of stocks that have that characteristic parasite infestation. The binary nature of the information makes it limited in resolution and unless multiple parasites are present and the infestation level varies between **a** number of the stocks of interest, or it is used in conjunction with some other stock identification method (see Wood et al. **1988),** only two groups can be identified. In many cases fish must be sacrificed to determine infestation levels. Thus, it is unlikely to be of significant value in separating treatment, replicate and control release groups. However, parasites may be useful in identifying supplemented from natural groups if either supplemented or natural fish are sheltered from some **parasite** that is present in the other population and the level of infestation can be determined from recovered carcasses. In addition, it could be possible that spring chinook rearing in the lower river (early outmigrants) are infested with parasites at a different level than fish moving out of the upper river as yearlings. For this reason, **a** subsample of spring chinook juveniles collected for GSI analysis of early and late outmigrants should also be screened for parasite infestation levels.

Momhometrics

Morphometrics has been suggested **as** a method to differentiate naturally occurring substocks, identify resident trout and steelhead, and to separate hatchery from natural smolts. Morphological differences between substocks of salmonids have been identified **(Beacham** and Murray 1987, **Beacham** et al. 1988; Winans 1984, 1987a) and can in some **cases** accurately separate groups of fish (Winans 1984). Typically, the accuracy of **substock** identification is not great enough to separate populations in a mixed sample. The task of identifying substocks of fish during outmigration, however, is made difficult by 1) the significant morphological changes occurring to fish during smoltification (Winans 1987b) and 2) morphological differences that develop

between fish of the same substock exposed to distinctly different rearing environments (Currens et al. 1989). Morphological changes occurring during smoltification are great enough that they will likely mask, shift, or confound genetically based substock differences (G. Winans, NMFS, pers. comm., 1989). Both resident and nonresident juvenile steelhead go through a process similar to smolting including silvering and ATP-ase level changes (K. Currens, OSU, pers. comm., 1989). Although morphological measurements have not been made to determine if the resident and nonresident fish go through similar body form changes while these physiological changes are occurring, it is likely that they do respond similarly. Such body changes associated with the smolting process will confound and alter any existing morphological differences between resident and nonresident fish.

Fin Clipping

Fin mutilation as a method of marking has a long history in the Columbia River (Rich 1927). Fins, other than adipose fins, can regenerate (Mears 1976), in some cases at rates as high as 46 to 53 percent in 3 months (Coombs et al. 1990). Increased mortality in fin-clipped fish has been reported as well (Nicola and Cordone 1973; Mears and Hatch 1976). The number of marks possible is relatively low unless more than two fins are removed. However, removing two or more fins will substantially increase the probability that survival, ability of fish to avoid predators and migratory ability are negatively affected. Also, when more than two fins are removed the decrease in fitness will not be equal across all marked groups resulting in biased experimental results. This technique is best applied in limited cases where a small number of release groups are needed and no other mark/tagging method is readily available. Fin-clipping may prove useful when used in concert with other marking techniques that provide additional information on release group. Fin-clipping's greatest strength is that it is a benignly recoverable mark that allows immediate identification of marked individuals.

SUMMARY RECOMMENDATIONS

♦ Any experimental study relying on recovery of adult PIT tags should be reevaluated in light of the tag shedding problem and hiatus in development of adult monitoring technology. Until the temporal trends in tag shedding are understood by species, adult return data from fish tagged as juveniles will be suspect. If adult monitoring technology is developed it will likely be based on newly developed lower frequency interrogation equipment making currently used 400 KHz based equipment obsolete.

♦ Research into benignly recoverable tags should continue. This would include V.I. tags in their various forms, body-area CWTs, and

adipose-fin injected CWTs. Applicability of body-area CWTs to identify juveniles should be investigated in order to determine if it is possible to accurately identify 15 or more release groups using a hand held CWT detector and if tagging multiple body areas has significant effects on behavior, survival and growth of fish.

♦ Elemental and fluorochrome marking studies should be continued to develop a method of applying marks to release groups that are benignly recoverable. In addition, planning should begin on developing a cross generation elemental mark that allows otoliths of the progeny of mature adult females to be marked prior to spawning. Such a mark would allow the reproductive success of females of known genetic background to be measured without applying a genetic mark.

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Appendix 6.

Definitions of Parameters in Conceptual Model for Tagging Evaluation Model - YKFP

N_i ≡ The number of tagged fish released from a treatment/control group **i.**

R_1 ≡ The estimated sampling or recovery rate at a particular monitoring facility, in this **case** the first **(i=1)** monitoring facility. Facilities will recover fish at different rates.

S_{ji} ≡ The survival rate from release to the $_j$**th** monitoring facility for the $_i$**th** release group. S_{ji} equals (number of recovered $_i$ **smolts/**R_j**)/**N_i**.**

$TAGC_k$ ≡ Tagging costs for tag type $_k$. Equals [N_i x (cost per tag type k)]. This will include associated costs for tagging equipment, trailer rental, temporary personnel, etc.

$TAGCAP_k$ ≡ Capital costs for **tag type k,** adjusted **(capitalized/amortized/expensed/discounted into future ?).**

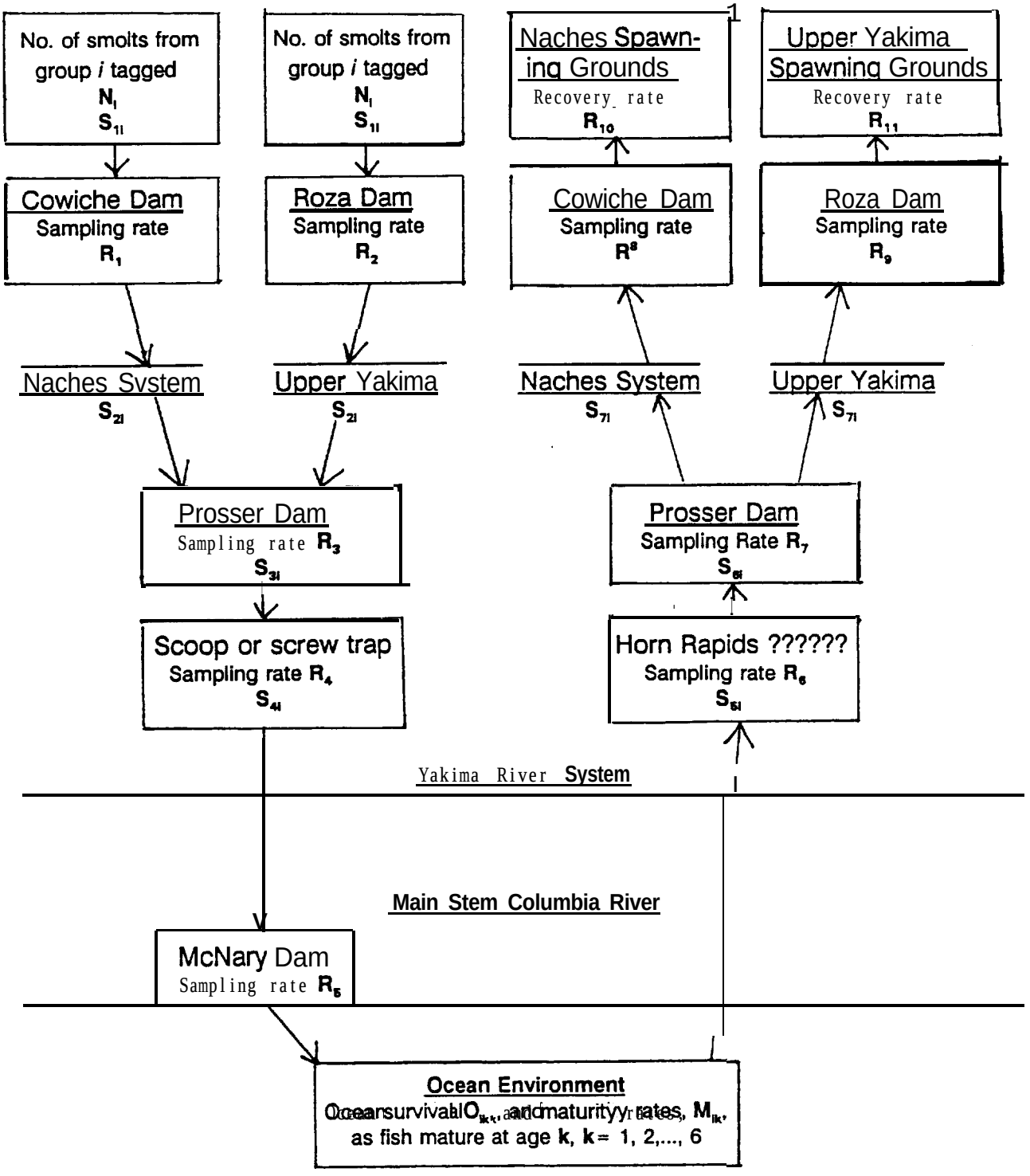
$TREC_k$ ≡ Tag recovery costs for tag type k. This includes any special handling, analytical costs, storage requirements, etc.

$TOTC_k$ ≡ Total costs for tagging N_i juveniles and recovering tags from juveniles and adults adults from one brood release.

$$TOTC_k = TAGC_k + TAGCAP_k + TREC_k.$$

Appendix 6. (cont.)

CONCEPTUAL MODEL FOR TAGGING AND RECOVERY EVALUATION - YKFP



REPORT NO. 3

EVALUATION OF JUVENILE AND ADULT MONITORING

- Evaluation of Mobile Downstream Migrant Trapping Gear in the Lower Yakima River
  by Dave Seiler

- Adult Trapping, Lower Yakima River, Fall 1990.
  by Dave Seiler

- Downstream Migrant Trapping in 1991 at Rosa Dam,
  by Dave Seiler

EVALUATION OF MOBILE DOWNSTREAM MIGRANT TRAPPING GEAR IN THE LOWER YAKIMA RIVER

INTRODUCTION

A major effort is underway to restore and enhance anadromous fish runs in the Yakima River basin. Such strategies as juvenile and adult passage improvement, flow control, habitat enhancement, and large scale supplementation with hatchery out-plants are planned and are being implemented. The supplementation program is a major experiment that, on the basis of monitoring and evaluation, will be altered as needed to achieve program goals. A critical component of this effort is the ability to measure production of out-migrant juveniles and returning adults. To provide part of this accountability, the Yakima Indian Nation (YIN) currently operates a number of monitoring stations throughout the basin (Fig.18). While these stations are located below the major spawning and rearing areas of spring chinook and steelhead, most of the fall chinook spawning is believed to occur downstream of Prosser Dam, where the lower-most juvenile enumeration facility is located.

Establishing downstream migrant monitoring capability near the mouth of the Yakima River is needed, not only to assess fall chinook production from natural spawners, but also to assess survival of other migrants to the lower river. Migration estimates made at the downstream migrant trap at Prosser indicate that juvenile salmonids experience significant instream mortality within the Yakima River. High predation rates, facilitated by extreme low flows at several points in the system, is the foremost hypothesis. Better quantification of this loss is required before remedial action is taken on this problem. Improving instream survival of migrants is critical to restoring the Yakima River's salmon and steelhead runs. In Spring 1989 and 1990, we assessed the feasibility of utilizing mobile downstream migrant trapping gear to capture juvenile salmonid emigrants from the lower Yakima River.

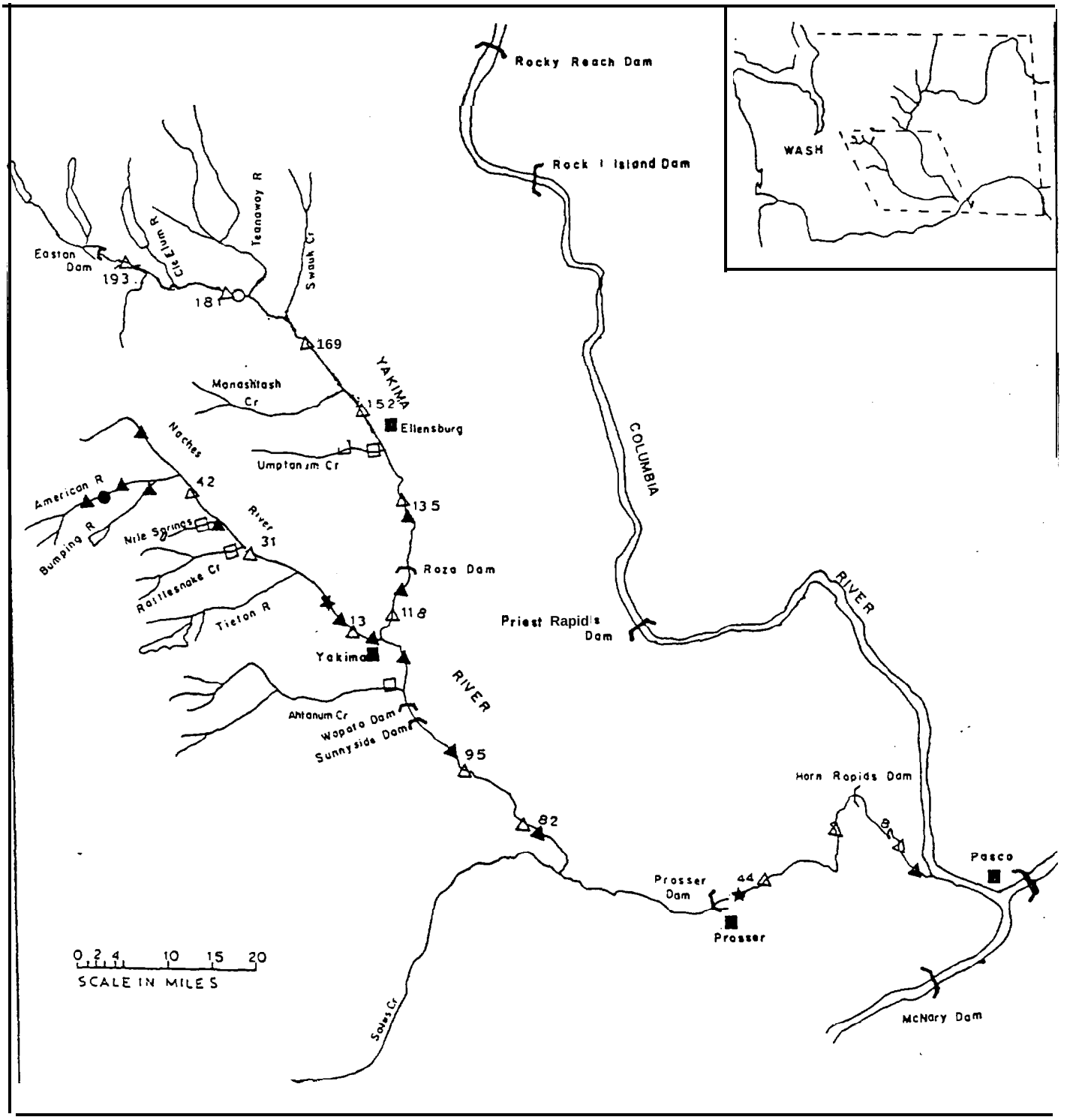


Fig.18. Map of the Yakima River Basin.

(Courtesy of the Yakima Indian Nation)

1989 TRAPPING AND GEAR OPERATION

We installed and operated a floating inclined plane screen trap, commonly referred to as a scoop trap **(Fig.19).** We have employed this gear extensively in many river systems over the last ten years, primarily to measure **coho** smolt production (Seiler et. al. 1981 and 1984). In order for this trap to capture downstream migrants, water velocity through the trap must exceed the migrant's swimming speed. As swimming speed is a function of fish length, inadequate velocity results in capture bias; the smaller and/or weaker individuals are captured at higher rates than larger and stronger migrants.

In April, we surveyed the lower Yakima River for a suitable trap site. Important criteria included sufficient water velocity, access, and suitable structure for anchor cable attachment. Because the lower five miles of the Yakima River is inundated at times, we surveyed upstream of this zone. We selected a trap site below the Van Giesen Road bridge in West **Richland** (R.M. 7). This structure provided good anchorage and stream velocity here was as fast as any other site in the lower river.

We transported the trap to the river and assembled it on May 22. The following day we completed outfitting the trap, floated it downstream **1/4** mile, and attached the anchor cables to the bridge. The trap was positioned in the fastest water available, which was at a point approximately 200 feet downstream of the bridge and 30 feet off the right bank. Here, velocities measured 4 to 4.5 feet per second, marginally higher than at other locations across the channel.

Trap operation began in the afternoon of May 23, and continued throughout each night until June 15. In addition,* we operated the trap around the clock (24 hours) on eight days (May 24 to **31),** to determine within-day migration patterns.

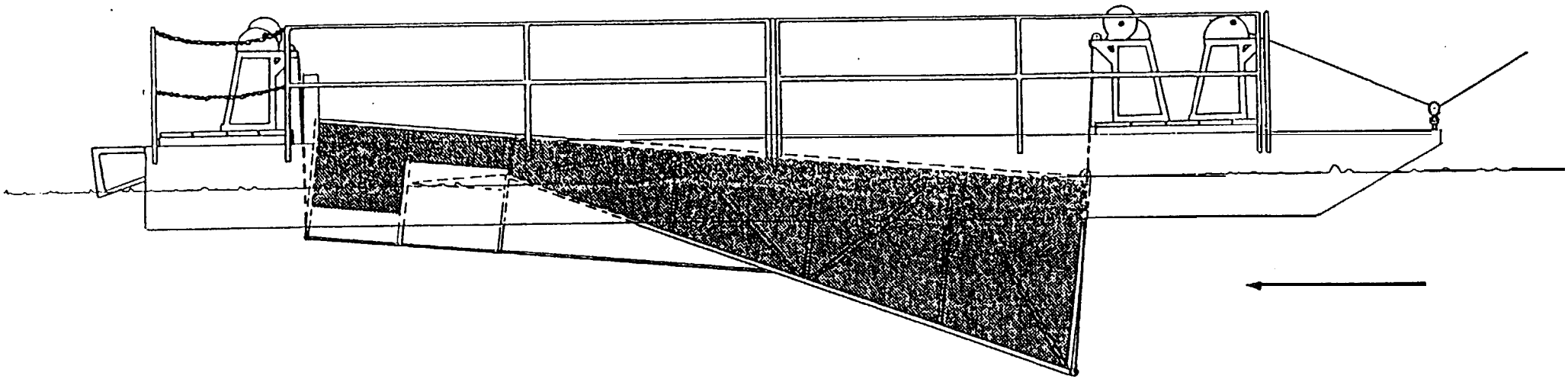


Fig.19. Side view of the floating inclined plane screen trap n the fishing position.

Over the trapping period, we captured 6,532 chinook smolts, the vast majority of which were fall chinook. Initial catches were less than 20 chinook per day, but increased in early June to the peak of 1,329 on June 9. Chinook catches steadily declined from this date through June 13, then increased over the last two days we fished (Fig.20). During the last 6 hours of fishing, from midnight until 0600 hrs on June 15, we caught 140 chinook, a marked increase over the previous night. To include this catch in **Fig.20,** we doubled it to account for the other half of the night not fished (dusk to midnight). We cannot estimate what portion of the migration had passed our trap by mid-June because we did not continue to fish throughout the month. However, the trapping operation conducted by the YIN at Prosser provides this fall chinook migration timing information. The YIN estimates that 62.5% of the total hatchery fall chinook migration had passed Prosser through June 14, and the migration was essentially over by the end of June (pers.comm. D. Fast, YIN). For estimation purposes, we will assume that by June **15,** 60% of the hatchery fall chinook migration was past our trap site.

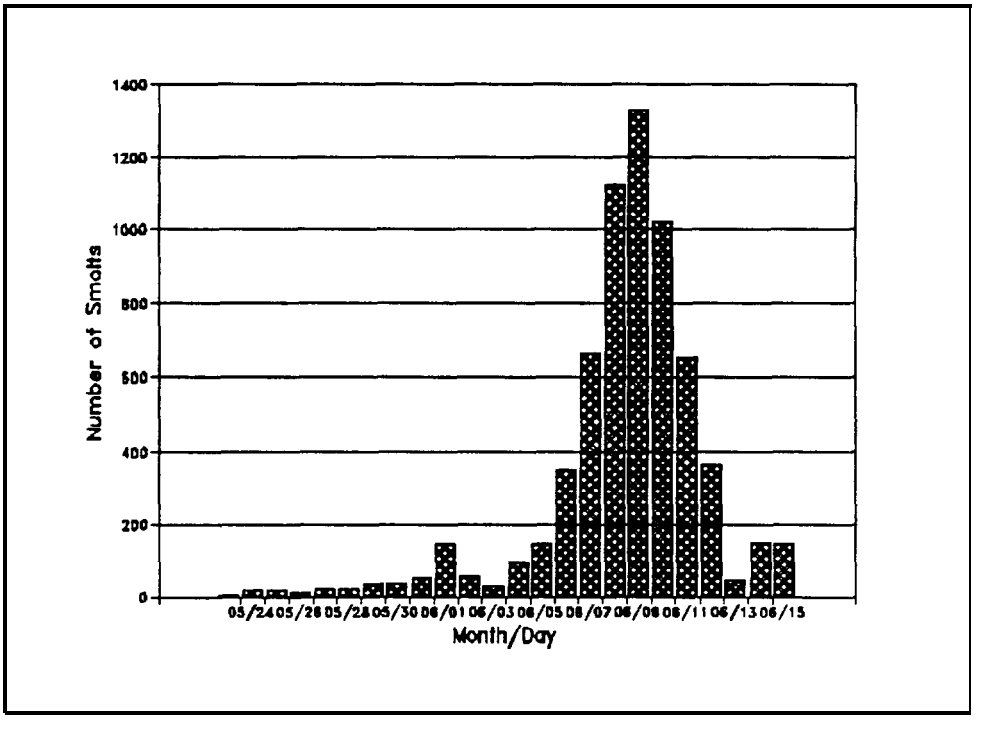


Fig.20. Chinook smolts captured at the Yakima River scoop trap, 1989.

In addition, we captured 17 coho smolts, 18 steelhead smolts, and numerous other non-salmonids.

Water temperature generally increased throughout the trapping period. In late-May, average daily stream temperature ranged around 61°F, and by mid-June, was over 70°F (Fig.21). Late afternoon and evening readings were highest, and early morning readings were the lowest within each day. The highest reading, 73°F, occurred on several occasions in mid-June. While these higher temperatures were clearly stressful, observed mortality was low. Over the season, 29 chinook died in the trap and 19 died during handling. Overall, direct (observed) mortality is estimated at 0.73% (48/6,532).

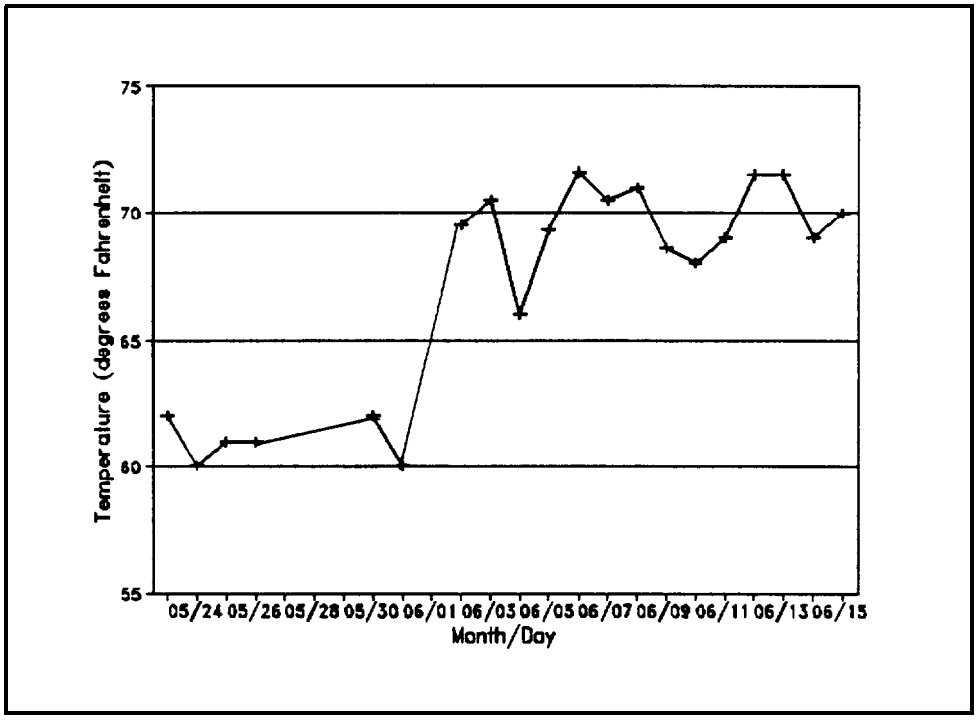


Fig.21. Average daily water temperature at West Richland (R.M. 7.0), Yakima River, 1989.

Each chinook captured was sampled for a missing adipose fin. Releases of ad-marked hatchery production first occurred on May 30, at Wapato Canal. Ad-marked chinook were first captured in the scoop trap five days later on the evening of June 4. From this date on, we captured 6,040 chinook, 2,051 of which were ad-marked (34%). As this mark rate is close to that estimated for the hatchery releases (35%; Table 35), it indicates that the chinook catch in June was almost entirely hatchery fish. Conversely, this high mark rate also

indicates natural production emigration between June 4 and June 15 was very low.

Table 35. Releases of fail chinook (Little White Salmon stock) in the Yakima River, 1989 (pers.comm., T. Scribner, YIN).

Date	Site	Number	Size (f/lb)	Number Tagged	Tag Code
5/30	Wapato Canal				
	Pen #1	120,174	72	102,148	51124
	Pen #2	100,153	88	85,130	51123
	Pen #3	119,794	75	101,825	51122
5/31	Prosser	200,204	157	200,204	51125
5/31	Prosser	176,453	141	———	
5/31	Sunnyside Dam	100,038	154	100,038	51126
6/1	Sunnyside Dam	100,039	153	100,039	51126
6/2	Prosser	356,020	141	———	
6/5	Prosser	355,033	133	———	
6/7	Horn Rapids	342,606	128	———	
Total		1,970,514		689,384	

We measured fork lengths on 756 chinook during the trapping period. Size ranged from 34 mm to 120 mm (Fig.22). As noted above, our catch was primarily hatchery fall chinook, which ranged in length from 60 mm to 97 mm, as represented by ad-marked fish (Fig.22). In comparison, size of presumed wild chinook (captured in May before the hatchery releases) ranged from 34 to 120 mm. No attempt was made to separate chinook into age classes. Extensive sampling by the YIN has demonstrated that spring chinook, which typically emigrate as yearlings, may range in size from as low as around 70 mm in June, but generally average over 100 mm. This length at age data indicates that we captured very few spring chinook. Our length sample included only 10 individuals over 100 mm. Consequently, our total catch of spring chinook was probably less than 100 fish. The reasons for this are two-fold: 1) the spring chinook smolt migration was largely over by late-May; and 2) the trap efficiency for the larger yearling migrants, due to relatively low velocities (4 to 4.5 fps). Velocities in excess of 6 fps through the scoop trap are required for unbiased capture of migrants measuring 100 to 150 mm. To reliably capture larger migrants such as steelhead smolts, velocity must exceed 7 or 8 fps.

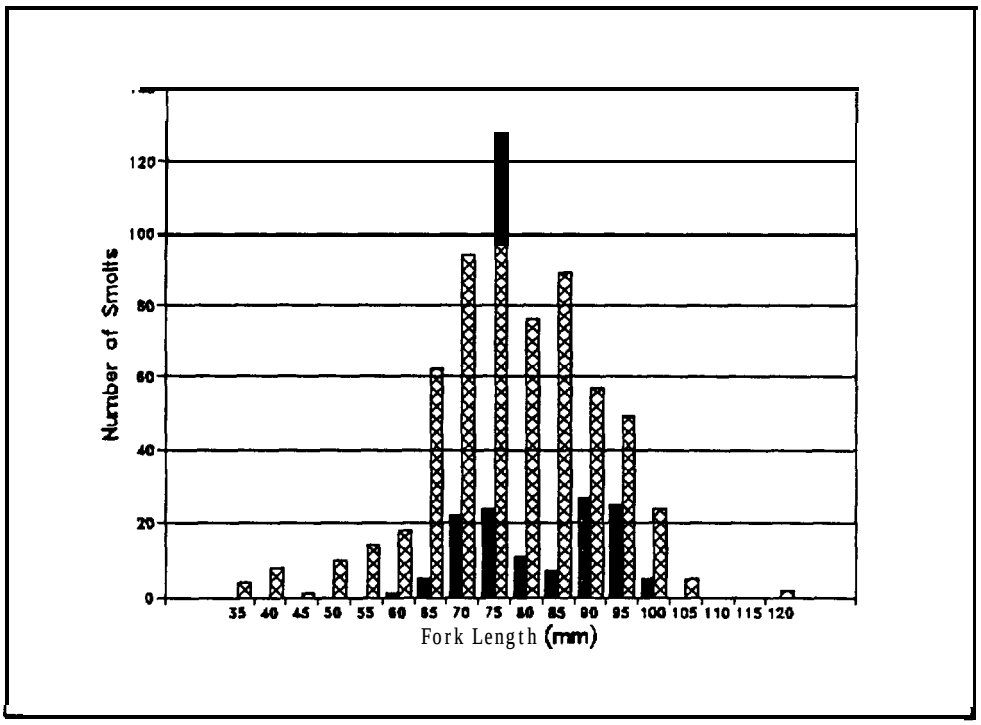


Fig.22. Fork lengths of ad-marked and unmarked chinook smolts measured at the Yakima River scoop trap, 1989.

The catches of very small chinook fry (34 to 45 mm) are of particular interest. This small size in late-May indicates extremely late wild spawning timing (December or January).

1989 Trap Efficiency

We had planned to estimate gear efficiency for fall chinook as soon as catches increased enough to provide an adequate number for marking and release upstream from the trap. Unfortunately, a critical component of this operation, our jet boat, was stolen on the night of May 25. We recovered the boat the next day, but the engine was damaged beyond immediate repair. Therefore, we conducted the efficiency tests by releasing marked groups from the river banks rather than across mid-channel as we had planned.

We released a total of 500 fin-marked chinook fingerlings over two nights (Table 36). Of these, we recaptured only 13 marks. It is obvious, however, that a strong bias occurred relating to the bank from which the marks were released. Of the 100 lower caudal "snips" (partial fin clips) released off the right bank, 200 yards upstream from the trap, we recaptured 11. Clearly, this group overestimates

capture efficiency because the trap was positioned closer to this bank. Conversely, we only recaptured 2 of the 400 fingerlings marked and released off the opposite bank, 1/4 mile upstream from the gear, and believe this underestimates gear efficiency. Had we been able to distribute the marked fry across the entire stream channel, trap efficiency would probably have been estimated at around 5% or less.

Table 36. **Scoop Trap efficiency tests, Yakima River, 1989.**

		RELEASE			RECAPTURE			
Date	Mark	Time	Location	Number	Number	Date	Time	Percent
6/6	UC	2350	LB	42				
6/7	UC	0320	LB	158				
	Total			200	2	6/7	0550	1.0
6/7	LC	2355	RB	68				
6/8	LC	0220	RB	32				
	Total			100	11	6/8	0307	11.0
6/8	UC	0250	LB	200				
	TOTAL			500	13			2.6

Another method of approximating trap efficiency is to compute the proportion of the river screened. The scoop trap entrance is six feet wide and six feet deep. At the trap site, the river is approximately 180 feet wide and, during the period we trapped, less than six feet deep. As we fished from the surface to the bottom, the ratio of trap width to river width (3.3%) would be a reasonable estimate of trap efficiency if several conditions were met:

1. Chinook were distributed evenly or randomly across the channel;
2. Chinook did not avoid or escape from the trap; and
3. The gear was fished continuously.

Although an exhaustive analysis of the degree to which these assumptions were met is not possible, based on our experience, we can predict the direction of the bias resulting from failure to meet each of these assumptions. Chinook fry probably do not distribute evenly or randomly across the entire stream channel. We positioned the trap where we expected migrants to be more concentrated, in the main flow, rather than in slower water near

the banks. If chinook were more concentrated where the trap was fished, as we believe they were, then we captured a higher proportion of migrants than the width ratio would predict. However, the failure to completely fulfill assumption number two offsets this to some degree. We did observe some chinook escaping from the trap entrance and from the live box. This was observed when heavy debris loads occluded the inclined screen, reducing velocity through the trap. Although the last assumption was not met either (because we did not fish continuously during daylight hours), extrapolating catches made during periods fished to periods not fished could easily correct for this. This would be a minor adjustment, however, as relatively few chinook were captured during daylight hours. An average of only 7% of the total catch was taken during daylight hours of the seven days fished 24 hours per day. Considering the opposing, though not necessarily equal, biases resulting from failure to fulfill conditions #1 and #2, we believe that an average efficiency of around 3% is reasonable. It is interesting to note that when the results of all three mark groups are pooled, trap efficiency is estimated at 2.6% (13/500).

1989 Instream Mortality

Even a rough estimate of trap efficiency enables us to evaluate the number of hatchery produced chinook fingerlings leaving the Yakima River and thereby assess instream mortality. Beginning May 30 and continuing through June 7, nearly 2 million hatchery-produced fall chinook fingerlings were released from various facilities upstream from the scoop trap (Table 35). These releases included an estimated 689,000 ad-marked chinook. At a trap efficiency of 3%, and assuming 60% of the migration past our trap by June 15, we should have caught around 35,000 hatchery fall chinook if they all survived to the lower river. Our catch of around 6,000 hatchery chinook indicates that the majority of the fish did not survive. While we do not precisely know our trap efficiency, estimates of instream mortality are significant when computed by even very low trap efficiency estimates. Based on a range of trap efficiencies, from a high of 5% to a low of 1% (2 percentage points above and below our point estimate of 3%), instream mortality of hatchery-produced fall chinook in the Yakima River in 1989 is estimated at 49-90% (Table 37). These estimates assume that 60% of the migration was past the trap site by mid-June.

Table 37. Estimates of bream mortality of hatchery-produced fall chlnook fingerlings in the Yakima River, 1969.

Trap Efficiency (%)	Estimated Number of Migrants Surviving To the Lower River [a]	Estimated Mortality (%)
1	1,000,000	49
2	500,000	75
3	335,000	83
4	250,000	87
5	200,000	90

[a] Catch (6,000)/trap efficiency x 0.6

The 34% ad-mark incidence in our catch of hatchery fall chinook indicates that survival to the lower river was similar for all releases. Chinook released above Prosser (aP) were marked at a rate of 90.6% (489,180/540,198) while those released below Prosser (bP) were marked at a rate of 14% (200,204/1,430,316). When the overall releases are pooled, the mark rate is estimated at 35% (689,384/1,970,514; Table 35). The apparent equivalent survival to the scoop trap of the aP and bP groups is significant, especially in light of the low survival (15%) to the Prosser trap of the aP groups (pers.comm. D. Fast, YIN). If the bP production had survived to the lower river at higher rates than the aP releases, as we would have expected, then the mark incidence in our catch would have been lower. Apparently, mortality per river mile on the bP groups was higher than that on the aP groups. If the estimate of mortality at Prosser of 85% is correct, then mortality on the bP group must exceed 90%. For example, if mortality on the aP group averaged 50% between Prosser and the scoop trap, then the bP groups also suffered an average mortality of 92.5% (1 - 0.5 x 0.15). At this survival rate, only about 150,000 hatchery chinook survived to the scoop trap. With this survival and assuming 60% migration during the period we trapped, trap efficiency is estimated at around 6.7% (6,000/150,000 x 0.6). As this rate is somewhat higher than our other estimates, either mortality was not this high, or trap efficiency is higher than we thought. If the mortality estimate at Prosser is correct, then scoop trap efficiency was higher than 3%. Regardless of the exact rates, however, it is apparent that instream mortality on hatchery fall chinook in the Yakima River in 1989 was very high.

While the low migration of wild fall chinook past our trap may be attributed in part to low escapement and poor survival to emergence, we believe that high instream mortality on fry is the major cause. This contention is based on the apparent loss of natural fall chinook migrants between the Prosser trap and our trap. At a trap efficiency Of 3%, our catch of around 500 wild chinook indicates less than 20,000 migrants passed the scoop trap

by mid-June. If this estimate represents about 60% of the migration, then the total migration past the scoop trap is around 30,000. By mid-June at the Prosser trap, over 85% (40,576) of the season total estimate of 47,598 wild fall chinook had outmigrated (pers.comm. D. Fast, YIN). Even if there was no production below Prosser, and assuming both of these estimates are reasonably accurate, these two migration estimates indicate mortality was near fifty percent between the two points of measurement. However, as the majority of the fall chinook spawning occurs below Prosser, instream mortality on the outmigrating offspring is much higher than 50%. We estimate total natural migration of fall chinook past the trap site at between 20,000 and 80,000 fry. These estimates are based on the following assumptions; trap efficiency ranged from 1% to 5%, around 60% of the wild chinook migration passed the scoop trap during the period we trapped, and around 500 of our total catch of 6,532 chinook fry were wild.

1989 CONCLUSIONS

1. Performance of the scoop trap was adequate for monitoring fall chinook production.

2. Because of low velocities in the lower Yakima River, velocity-dependent gear is not effective for capturing larger migrants such as spring chinook and steelhead smolts.

3. Instream mortality of hatchery-produced fall chinook was high. We estimate mortality in the range of 49 to 90%.

4. The number of naturally produced fall chinook outmigrants surviving to pass our trap was relatively low. We estimate between 20,000 and 80,00 wild chinook survived to the lower river.

1989 RECOMMENDATIONS

1. Better estimates of fall chinook production and survival.could be achieved with the scoop trap by commencing trapping earlier in the spring, continuing trapping throughout the migration, and more rigorously evaluating trap efficiency.

2. We recommend, however, that different gear, not dependent on high velocity, be deployed. If this can be accomplished, then, for a similar expenditure of manpower, in addition to measuring fall chinook production and survival, instream mortality of spring chinook and steelhead can also be assessed.

1990 TRAPPING GEAR AND OPERATION

Following our recommendations, we decided to test a mobile trap, that was not dependent on high velocities to capture a portion of all downstream migrants emigrating from the lower Yakima. Recently, a floating "screw" trap was developed in Oregon that functions in lower velocities. This gear traps fish using an auger that literally "screws" migrants into a live box (Fig.23). Two, four ft wide tapered flights, wrapped 360 degrees around a nine foot long shaft, form the basic trap. These flights are housed inside a mesh covered cone shaped frame. The shaft is aligned with the flow and is lowered to the water's surface via davits and winches mounted on two steel pontoons. This gear fishes half of an eight foot circle, a cross sectional area of 25 square feet.

Water current acting on the flights causes the trap to rotate. With every 180 degrees of rotation, a flight enters the water while the other emerges. As the leading edge of a flight emerges from the water it prevents the escape of any trapped migrants. The fish are gently augured into a solid sided and baffled live box. A small drum screen located at the rear of the live box removes organic debris, the nemesis of all fish traps and other screening devices. The main shaft drives the drum via belts and a 90 degree gear reducer.

We built a screw trap prior to the 1990 season for installation at the West Richland site, where we had operated the scoop trap in 1989. We transported the trap to the river and assembled it on April 23. On April 24, we began fishing and technicians from the National Marine Fisheries Service (NMFS) outfitted the trap with two PIT tag detectors. One detector was battery (DC) powered, while the other ran off a portable generator (AC). We passed all captured salmonids through this dual PIT tag detection system which automatically recorded the information in the computers.

To assess capture efficiency, we released nine groups of marked fall chinook upstream of the trap between May 21 and June 6. Smolts captured in the trap were marked with either an upper or lower partial caudal fin-clip, taken upstream various distances (from 1/2 mile to 4.8 miles), and released. In addition to these groups, 499 branded and PIT tagged fall chinook were released at Prosser on May 22. *Over* the season, 20,000 PIT tagged smolts (sockeye, steelhead and spring and fall chinook) were released into the Yakima River at various points upstream of West Richland. This report does not include an analysis of the recoveries of these tags in the screw trap because NMFS is currently performing this task.

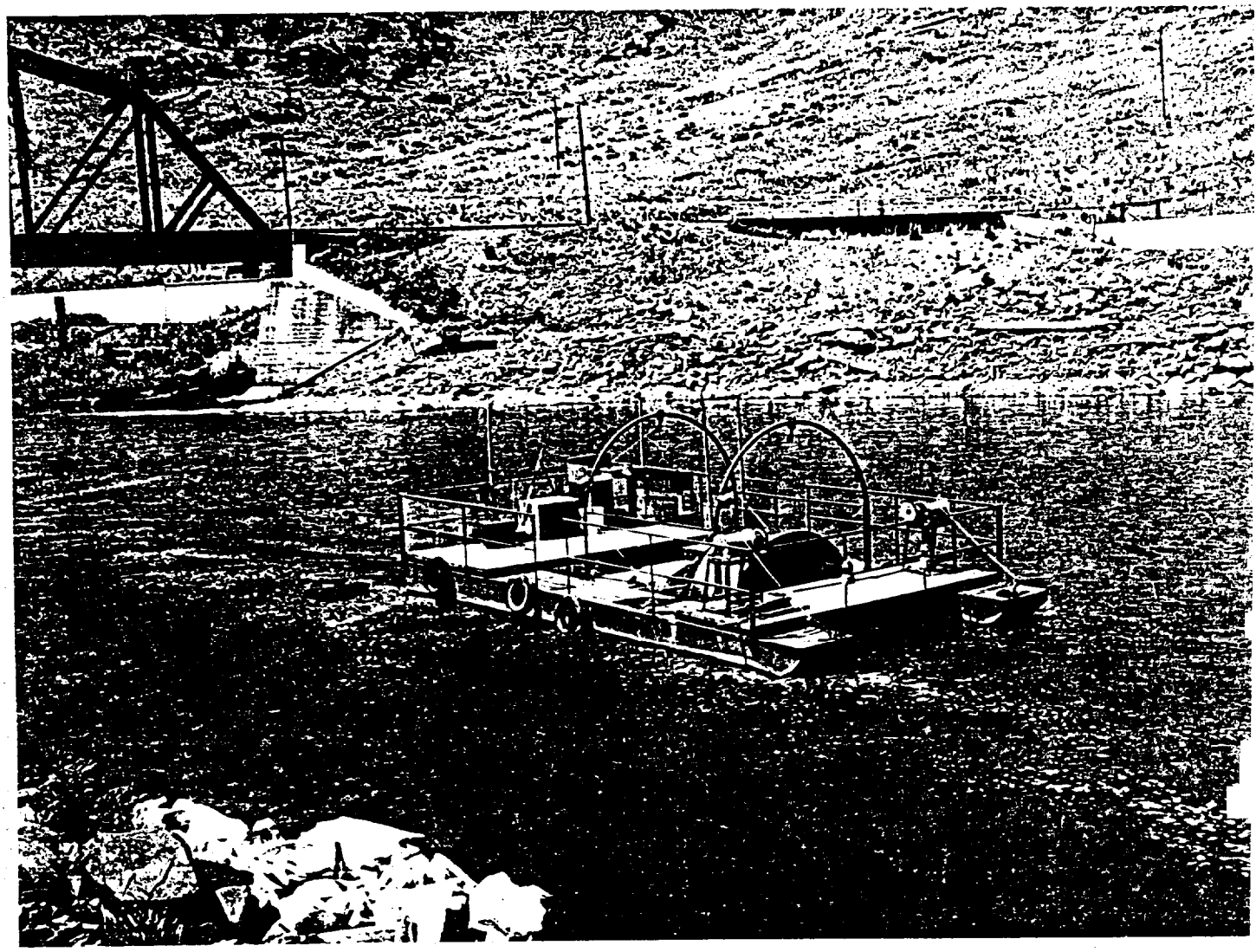


Fig.23. Floating screw trap in operation

1990 RESULTS

Trap operation

Discharge was higher during the 1990 trapping period than in the previous year (Fig.24). This produced stream velocities at West Richland averaging around 6 fps, significantly higher than in 1989 when maximum water velocity was only 4.5 fps. At 6 fps the screw turned at around 12 rpm. Over the season, rotation speed ranged from a low of around 8 rpm to a high of 14.5. This variation resulted not only from discharge but also from the lateral placement of the trap in the channel.

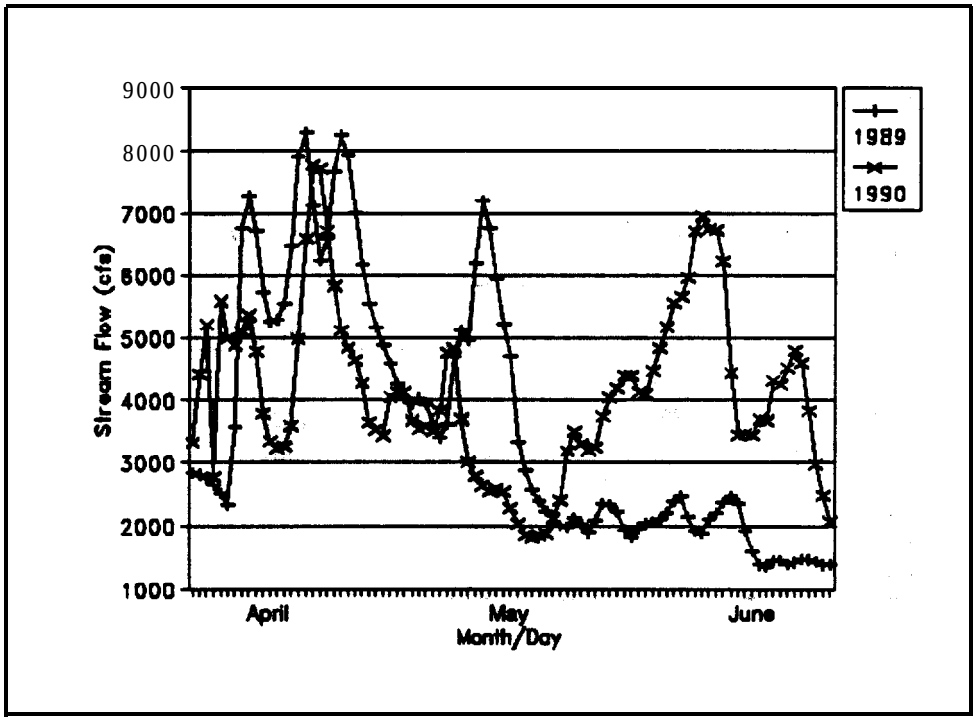


Fig.24. Discharge of the Yakima River at Kiona, Spring 1989 and 1990.

The self cleaning drum in the live box, enabled nearly continuous operation. On two occasions during the season, however, (April 30 and May 29) the aluminum flights broke. Each time, we removed the screw assembly from the trap and repaired the damage. When the flights broke a third time, late in the season on June 10, we terminated the operation. As constructed, these flights were clearly not strong enough to sustain continuous operation in 6 fps water. The shallow pitch on the flights contributed to this

problem. The flights made 60 degrees of rotation in the first three feet of shaft length, 120 in the next three feet, and 180 in the last three feet. With this pitch the trap turned approximately two rpm for each fps of velocity. This would be a good configuration in the water velocities observed in 1989 (around 4 fps or less). In the higher velocities encountered in the lower Yakima River in 1990, however, the load placed on the flights was excessive.

Except for breaking the weak flights and resultant down time, we operated this gear most of the trapping period with no other problems. In total, the gear operated 79% of the time during the trapping period, April 24 through June 10 (Table 38). The drum in the live box removed all the small organic debris enabling continuous operation and, as a result, virtually no trap maintenance was required. On several occasions, however, large woody debris jammed in the trap and stopped the screw. The only routine demands on the two man crew involved processing the catch, each morning and evening.

Table 38. Summary of screw trap operation on the Yakima River, from April 24 at 1900 hours to June 10 at 2000 hours.

	APRIL	MAY	JUNE	TOTAL
Total hours	149	744	236	1,129
Hoursfished	73	611	203	667
% of time fished	49	82	86	79
# of nights	7	31	9	47
# of nights fished	8	30	8	44
# days	6	31	10	47
# days fished	0	20	9	29

Downstream Migrants Captured

Unlike the velocity dependent scoop trap, the screw trap captured a portion of all juvenile salmonids migrating from the lower Yakima River including large smolts such as steelhead in excess of 300 mm (Table 39).

Table 39. Species captured In the Yakima River screw trap, 1990.

Salmonids		Non-salmonids	
Species	Number	Species	Number
Coho	1,078	Largemouth Bass	1
Steelhead	684	Smallmouth Bass	92
hatchery	181	Bluegill	2
wild	483	Carp	4
Sockeye	10	Brown Bullhead Catfish	15
Fall Chinook	18.889	Channel Catfish	10
Spring Chinook	778	Chiselmouth	59
Mountain whitefish	203	Longnose Dace	16
		Lamprey	1
		Mosquitofish	2
		Peamouth	2
		Sandroller	2
		Sucker	54
		Sunfish	31
		Tadpole	127

Fall chinook were the most abundant migrant captured over the trapping period (Table 39). The total catch of 18,889 is a mix of wild and hatchery smolts. Catches of wild fall chinook were low during late April through mid May. Following YIN/USFWS releases of hatchery fall chinook in mid-May (Table 40), wild fall chinook were indistinguishable from the unmarked hatchery fish. Ad-marked fall chinook composed 7.4% of the catch (1,388/18,889). On the basis of their larger size, we identified 778 chinook as yearlings, presumably wild spring chinook.

Table 40. Releases of fall chinook (Little White Salmon stock) in the Yakima River, 1990 (pers.comm. T. Scribner. YIN).

Date	Site	Number	Size	#CWT	Taa Code
5-18-90	Wapato Canal				
	Pen #1	79,830	105	72,271	5-I-1-2-10
	Pen #1	79,860	105	0	
	Pen #2	80,103	102	79,141	5-I -1-2-9
	Pen #2	79,900	102	0	
	Pen #3	80,135	102	79,173	5-I-I-2-8
	Pen #3	79,906	102	0	
5-15-90 [a]	Parker	79,892	180	79,413	5-I -1-2-11
5-15-90	Parker	20,088	180	0	
5-17-90	Parker	100,000	221	0	
5-14-90	Prosser [b]	38,151	206	39,113	5-I-I-2-7
5-14-90	Prosser	42,589	206	42,546	5-21-20
5-14-90	Prosser	271,124	178	0	
5-14-90	Prosser	88,480	209	0	
5-16-90	Prosser	440,000	108	0	
5-18-90	Benton City	438,000	221	0	
	TOTAL	1,998,058		390,657	

[a] The transport truck broke down for 2.0 hours; tank temperature climbed from 48 to 55 degrees F. Fish were very stressed at release.
[b] Below Prosser Dam

Virtually all of the 1,078 coho smolts caught were hatchery produced. Sixty nine of the coho smolts were ad-marked, an incidence of 6.4%.

The catch of 664 steelhead smolts included 181 hatchery smolts (180 were ad-marked) and 483 wild fish. Fork length of 29 wild steelhead selected at random averaged 203.6 mm and ranged from 149 to 295 mm with a standard deviation of 43.2 mm. One steelhead smolt had an external numbered wire tag.

Various non-salmonids were also caught (Table 39). On occasion, predation in the live box, primarily by smallmouth bass (94 caught) was evident. The 31 sunfish we captured also probably preyed upon fall chinook smolts in the trap to some extent. It is interesting to note that not one squawfish was captured.

Upon removal from the trap, fish were in excellent condition and observed mortality was very low. Over the season, mortalities on salmonids totaled only 1 coho smolt, 2 spring chinook smolts and 56 fall chinook. The largest single incident of mortality occurred when a log jammed in the rotating screw and some chinook were impinged on the screen. Other mortalities appeared to result largely from damage by predators and the thrashing about of large fish such as suckers, whitefish, bass, catfish and carp in the live box.

Estimation of Fall Chinook Prod-

In late-April and during the first half of May, catches of wild fall chinook were so low that we had to wait until the hatchery fish arrived in mid-May to begin testing trap efficiency (Fig.25). In total, we released 3,095 fin-marked smolts in nine groups and recaptured 170 for an average recapture *rate* of 5.3% (Table 41). This ratio underestimates trap efficiency, however, because release groups 3 and 6 were flawed. Of the other seven presumed unbiased test groups, efficiency ranged from 3.2 to 11.6% and averaged 7.3% (154/2,109) (Table 41). One group of PIT-tagged fall chinook released at Prosser was captured at a rate of 4.2% (Table 42). While this rate is within the range we measured, it is an underestimate of trap efficiency by whatever mortality occurred between Prosser and West Richland. Travel time between Prosser and West Richland is estimated at around five days by this group.

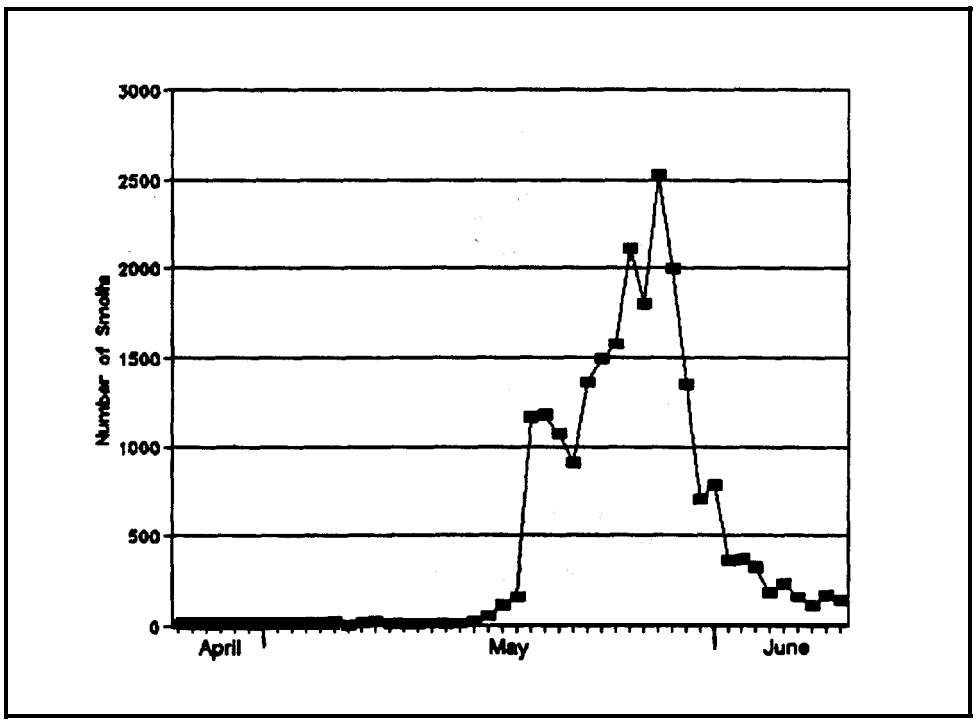


Fig.25. Projected fall chinook smolt catch at the Yakima River screw trap, April 24 to June 10, 1990.

Table 41. Screw trap efficiency tests with fall chinook, Yakima River, 1990

	RELEASE					RECOVERY			
Test#	Date	Mark	Time	Location	Number	Number	Date	Time	Percent
I.	5/21	UC	2045	a	113	9	5/21	2400	
	5/22	UC	0005	b	317	14	5/22	0800	
			TOTAL		430	23			5.35
II.	5/22	LC	2000	c	187	4	5/23	2345	
	5/23	LC	0015	c	225	11	5/23	0900	
						3	5/23	2100	
			TOTAL		392	18			4.59
III.	5/25	UC	1015	c	480 [d]	5	5/26	0300	2.08
IV.	5/26	LC	2030	b	250	53	5/26	2145	
	5/26	LC	2120	b	250	4	5/27	0845	
						1	5/28	0930	
			TOTAL		500	58			11.80
V.	5/27	UC	1540	b	450	35	5/27	2115	
						1	5/28	0930	
						2	5/28	2200	
			TOTAL		450	36			8.44
VI.	5/30	LC	1435	b	506	11 [e]	5/30	2115	2.17
VII.	6/5	UC	0845	b	62	2	6/5	2030	3.22
VIII.	6/6	LC	1725	b	175	11	6/6	2015	8.28
IX.	6/6	UC	1715	b	100	4	6/7	2000	4.00
			GRAND TOTAL		3095	170			5.30

[a] 3/4 mile upstream from trap.
[b] 1/2 mile upstream from trap.
[c] Twin Rivers Bridge, 4.8 miles upstream from trap.
[d] Low DO levels from tank over-crowding caused extreme stress.
[e] A log was removed from the trap at 2000 hrs. Some fish may have escaped.

Table 42. Recovery of branded fall chinook *, Yakima River screw trap, 1990.

Date	T M	# of brands
05-27	1530	11
05-27	2115	5
05-28	0930	1
05-28	2200	2
	TOTAL	19

* At 0930 hrs May 22, 499 branded and pit tagged fall chinook were released at Prosser. Twenty-one PIT tags were detected, indicating that we missed 2 brands.

In 1989, we used migration timing estimates generated at the Prosser smolt trap to approximate the proportion of the fall chinook migration past West Richland during the period we trapped. This information estimated that 60% of the migration had passed our trap by June 15. In 1990, however, estimates made at Prosser indicated that by June 10, the date we stopped trapping, only 30% (89,208/293,084) of the total migration had passed that point. Assuming this estimate is also correct for the migration past West Richland, we estimate that approximately one million fall chinook emigrated from the lower river.

This estimate was generated using the following procedures:

1. Catch per hour rates were computed for day and night periods fished (Table 43 and 44). We applied the appropriate catch rates to the 242 hours not fished during the trapping period to estimate the number of fall chinook we would have caught had we fished during these periods (Table 45). Adding this expected catch (3,990) to the actual catch, we would have caught 22,879 fall chinook had we fished continuously from April 24 through June 10.

2. We applied the average estimated trap efficiency of 7.3% to this projected catch to calculate around 300,000 fall chinook passed the trap site before we stopped fishing on June 10.

3. Assuming 30% of the total migration past West Richland occurred by June 10, then we estimate total fall chinook production at around one million fish.

Table 43. Comparison of day and night catches of fail and spting chinook smolts In the Yakima screw trap, May 1 to May 15, 1999.

Date/Interval	Hour	Night				Da			
		Fall		Spring		Fall		Spring	
		catch	Catch/ Hour	Catch	catch/ Hour	Catch	catch/ Hour	Catch	Catch/ Hour
May 1 @ 1945 hrs - 0715 hm May 2	11.5	4	0.35	8	0.70				
May 2 @ 0900 hrs - 2000 hrs	11.0					6	0.73	4	0.38
May 2 @ 2000 hrs - 0800 hrs May 3	120	7	0.58	7	0.56				
May 3 @ 0800 hrs - 2000 hrs	12.0					6	0.50	1	0.63
May 3 @ 2000 hrs - 0830 hrs May 4	12.5	10	0.80	2	0.16				
May 4 @ 0830 hrs - 1930 hrs	11.0					5	0.45	4	0.38
May 4 @ 1945 hrs - 0830 hrs May 5	12.75	16	1.41	16	1.25				
May 5 @ 0845 hrs - 2000 hrs	11.25					7	0.62	2	0.16
May 5 @ 2015 hrs - 0800 hrs May 6	11.75	0*		0*					
May 6 @ 0800 hrs - 1200 hrs	12.0					2	0.16	1	0.63
May 6 @ 2000 hrs - 0800 hrs May 7	12.0	2	0.17	3	0.25				
May 7 @ 1815 hrs - 0800 hrs May 8	13.75	21	1.53	28	1.89				
May 8 @ 1700 hrs - 1830 hrs	1.5					5	3.3	7	4.67
May 8 @ 2100 hrs - 0700 hrs May 9	10.00	1	0.10	14	1.4				
May 9 @ 1915 hrs - 0730 hrs May 10	1225	3	0.24	7	0.57				
May 10 @ 0830 hrs - 1930 hrs	11.0					5	0.45	1	0.09
May 10 @ 1930 hm - 0715 hm May 11	11.75	2	0.17	28	2.21				
May11 @ 2000 hrs - 0800 hrs May 12	12.0	4	0.33	39	3.25				
May 12 @ 2000 hrs - 0700 hrs May 13	11.0	3	0.27	24	2.18				
May 13 @ 0800 hrs - 1930 hrs	11.5					0		7	0.61
May 13 @ 2000 hrs - 0730 hm May 14	11.5	9	0.76	31	2.70				
May 14 @ 0800 hrs - 2030 hrs	12.5					3	0.24	10	0.80
May 14 @ 2030 hrs - 0730 hrs May 15	11.0	9	0.82	18	1.64				
Mav 15 @ 0730 hm - 2015 hrs	12.75					8		8	0.47
TOTAL Night	154	93	0.5039	221	1.4351				
Dav	95					49	0.5156	43	0.4526

. Two bass were In the live box and may have eaten the catch.

Table 44. Comparison of day and night catches of Fall and Spring chinook smolts in the Yakima River Screw Trap, May 15 to June 5, 1990

Date/Interval	Hour	Night				Day			
		Fall		Spring		Fall		Spring	
		catch	Catch/ Hwr	Catch	Catch/ Hour	Catch	Catch/ Hour	Catch	Catch/ Hour
May 15 @ 2015 - 0630 May 16	12.25	26	2.29	16	1.31				
May 16 @ 0900 - 2100	12.0					12	1.00	0	
May 16 @ 2100 - 0745 May 17	10.45	64	6.12	23	2.20				
May 17 @ 0745 - 2030	12.75					28	2.20	6	0.47
May 17 @ 2030 - 0900 May 18	12.5	140	11.26	33	2.64				
May 18 @ 0900 - 2000	11.0					29	2.64	4	0.36
May 18 @ 2000 - 0830 May 19	12.5	257	20.56	35	2.60				
May 19 @ 0830 - 2000	11.5					193	16.78	0	
May 19 @ 2000 - 0700 May 20	11.0	1,863	169.36	52	4.73				
May 20 @ 0700 - 1945	12.75					77	6.04	2	0.16
May 20 @ 1945 - 0930 May 21	13.75	1,214	88.29	36	2.62				
May21 @ 0930 - 2015	10.75					116	10.79	2	0.19
May 21 @ 2015 - 0800 May 22	11.75	809	68.85	27	2.30				
May 22 @ 0800 - 1930	11.5					171	14.67	7	0.61
May 22 @ 1930 - 0900 May23	13.5	942	69.76	24	1.78				
May 23 @ 1415 - 2100	6.75					296	43.85	10	1.46
May 23 @ 2100 - 0945 May 24	12.75	583	45.73	35	2.75				
May 26 @ 0830 - 2145	13.25					1,040	7a.49	14	1.06
May 26 @ 2145 - 0845 May 27	11.0	a82	80.18	a	0.73				
May 27 @ 0645 - 2115	12.5					a69	69.52	20	1.60
May 27 @ 2115 - 0930 May 28	12.25	1,023	63.51	13	1.06				
May 29 @ 1730 - 2145	4.25					566	133.16	6	1.41
May 29 @ 2145 - 0730 May 30	9.75	434	44.51	7	0.72				
May 30 @ 0730 - 2115	13.75					961	69.89	7	0.51
May31 @ 0830 - 2100	12.5					546	43.84	6	0.48
May 31 @ 2100 - 0600 June 1	11.0	151	13.73	0					
June 1 @ 0800 - 2030	12.5					620	49.60	1	0.08
June 1 @ 2030 - 0900 June 2	12.5	211	16.66	1	0.08				
June 2 @ 0900 - 2030	11.17					167	16.74	2	0.18
June 2 @ 2030 - 0200 June 3	5.5	34	6.16	1	0.18				
June 3 @ 0200 - 2030	19.5					357	18.31	9	0.46
June 3 @ 2030 - 0630 June 4	12.0	129	10.75	0					
June 4 @ 0630 - 2030	12.0					177	14.75	1	0.08
June 4 @ 2030 - 0630 June 5	12.0	62	5.17	1	0.08				
June 5 @ 0830 - 2030	12.0					112	9.33	0	
TOTAL Night	197	6,826	44.86	312	1.56				
Day	211					6.359	30.08	97	0.46

Table 45. Estimated catch of spring and fall chinook during hours not fished, Yakima River screw trap, April 24 to June 10, 1990.

Date/interval		Hours Not Fished	Night				Da			
			Fall		Spring		Fall		Spring	
			Est. Catch	catch/ How	Est. Catch	catch/ Hour	Est. catch	catch/ Hour	Est. Catch	catch Hour
April 24 - April 30 night		3.75	2.25	0.60	5.40	1.44				
April 24 - April 30 day		72.08					37.48	0.62	32.44	0.45
May1 - 15 night		3.25	1.95	0.60	4.68	1.44				
May 1 - 15 day		76.0					39.52	0.52	34.20	0.45
May 16 @ 0830 - 0900		0.5					0.50	1.60		0
May 23 @ 0900 - 1415		5.25					230.21	43.65	7.77	1.46
May 24 @ 0945 - 1740		7.92					347.29	43.85	11.72	1.48
May 24 @ 1810 - 2045		2.58	118.04	45.75	7.10	2.75				
May 25 @ 0800 - 1730		9.5					745.66	78.49	10.07	1.06
May 28 @ 2200 - 0730 May 29		9.5	793.35	83.51	10.07	1.66				
May 29 @ 0730 - 1730		10.0					1,331.80	133.18	14.10	1.41
May31 @ 0015 - 0830		8.25	113.27	13.73		0				
June 2 @ 1300 - 1326		0.33					5.52	16.74	0.33	1.00
June 8 @ 2100 - 0830 June 9		11.5	80.50	7.00		0				
June 9 @ 0830 - 1800		9.5					66.50	7.00	0.95	0.10
June 9 @ 1800 - 0500 June 10		11.0	77.w	7.00		0				
TOTAL	Night	43.83	1,186	25.28	27	0.49				
	Day	197.08					2,804	14.46	112	0.63

Wild Production. A basic problem in estimating wild fall chinook production in the Yakima River is the large release of unmarked hatchery fish. Before the hatchery fish appeared (May 16), catches of presumed wild chinook were low. This early portion of the migration, however, cannot be used to project the entire run because it is too small a component of the total production and, more importantly, because hatchery fish have been planted every year, the migration timing for wild chinook is not known. Therefore, our estimate of about one million fall chinook migrating past West Richland is for hatchery and wild fish combined.

Assuming that the mortality estimated on hatchery fish released above Prosser is correct and, further, that fish released below Prosser experienced similar mortality, then the total number of hatchery fish surviving to the lower river can be approximated. Survival above Prosser is estimated at 43% (293,000/680,000) and if it is 50% to West Richland, then 'around 147,000 of these fish survived to the lower river. If mortality was around 50% on the 1,318,000 fish released below Prosser, then around 660,000 of these survived to the lower river. If these estimates are approximately correct, then around 800,000 hatchery fish survived to West Richland. Further, if our estimate of around a million fall chinook migrating from the lower river is approximately correct, then, by subtraction, it appears wild chinook accounted for around 200,000 migrants.

Although we do not place a lot of confidence in this estimate because it is based on so many unsubstantiated assumptions, it appears to be about what' could be expected given the level of natural spawning. In 1989, 636 adultfall chinook were passed over Prosser Dam. This component of the run is believed to compose about a third of the total. If this is. true, then the total escapement was around 1,900 fish. If half were females and fecundity averaged around 4,500 eggs, then total deposition was around' 4.3 million eggs. Further, at an average survival to emergence of 10% and then to emigration from the system of 502, around 200,000 wild chinook fingerlings would be produced.

Trap Efficiency on Yearling Chinook Smolts

The 1990 trapping effort focused on fall chinook and as a consequence, began after the peak migration of yearling chinook occurred. We did capture an estimated 778 yearling chinook. This number has to be qualified as an estimate because there is some overlap in size between small spring chinook and large fall chinook. We used the following size criteria to separate these two stocks:

4-25 to 5-14 fish 60 mm or less were considered falls
5-15 to 5-19 fish 80 mm or less were considered falls
5-29 to 6-10 fish 100 mm or less were considered falls

We estimate that, had we fished continuously during the period trapped, we would have caught an additional 139 smolts (Table 45). Relating the projected catch of 917 to the 32,882 smolts the YIN estimated past Prosser after April 19 indicates our trap efficiency on these migrants averaged around 2.8%. April 19 was selected because PIT tag recoveries at McNary Dam indicated that migration out of the system occurred within a week. The accuracy of this estimate relies on the estimate at Prosser and the assumption that no mortality occurred between Prosser and West Richland. If less than 33,000 yearlings passed our trap, then gear efficiency was higher than 2.8%. Efficiency varies as a function of discharge and position of the trap in the channel. The general decline in catches near the end of the period trapped, however, seems to indicate that the daily catch generally reflects abundance (Fig.26).

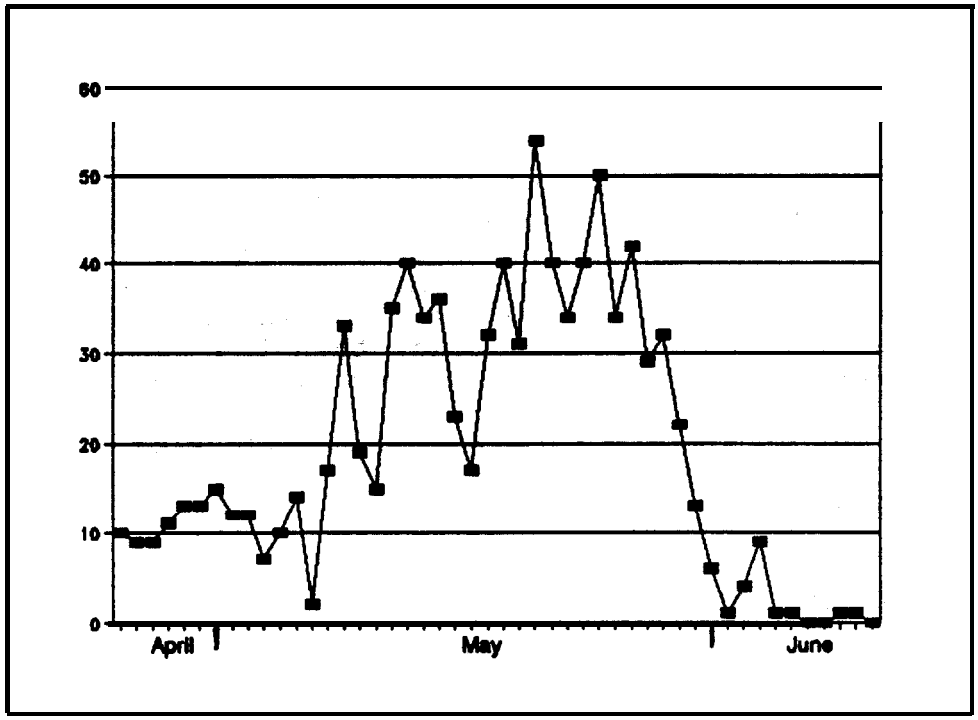


Fig.26. Projected spring chinook smolt catch at the Yakima River screw trap, April 4 to June 10, 1990.

1990 CONCLUSIONS

1. The screw trap performed well., capturing a portion of all species/ages of juvenile salmonids emigrating from the lower Yakima River.

2. Capture efficiency for fall chinook was estimated at around 2%.

3. Around 700,000 - 800,000 hatchery chinook survived to emigrate past West Richland.

4. Wild chinook production was estimated at around 200,000 to 300,000 migrants.

5. Capture efficiency on *larger* migrants (spring chinook, steelhead and coho smolts) was not estimated but based on the mean size of these migrants captured, it appears that this gear is not size selective.

1990 RECOMMENDATIONS

1. Stronger flights set at a steeper pitch would alleviate the only problem encountered with this gear in 1990.

2. Better estimates of fall chinook production could be obtained by fulfilling these recommendations:

   a. Operating the trap throughout the emigration.

   b. Estimating effidisncy throughout the season with releases of marks and, thereby developing a relationship between flow and efficiency.

   c. Marking all hatchery reieases to enable estimation of wild chinook production and also survival of hatchery fish from the lower river.

3. Estimate the capture efficiency for other migrants via release of mark groups to enable estimation of instream loss.

4. Measure the fork length of a sufficient sample of the yearling migrants captured in this gear to enable comparison with sizes measured at Prosser. Test for size selection by the screw trap.

ADULT TRAPPING, LOWER YAKIMA RIVER, FALL 1990

INTRODUCTION

Estimation of the numbers of fall chinook spawners in the Yakima River has never been accomplished, primarily because of poor visibility. Although the adults passing Prosser Dam are counted, most of the run is believed to spawn downstream of this point. Therefore, YKFP planners have identified the development of adult escapement estimation techniques for fall chinook returns to the Yakima River as a priority pre-facility need. During Fall 1990, we conducted a very small scale field test of one unit of mobile temporary adult trapping gear in the lower Yakima River.

TRAPPING GEAR

Commonly called hoop traps, this gear was developed and used to capture adult chinook salmon in the Sacramento River (Hallock et. al. 1957). We routinely use this gear to capture coho in the lower mainstems of large river systems for adult tagging studies. Ten foot diameter hoops (1 inch steel pipe) are spaced three feet apart to form a 20 foot long cylinder comprise the basid shape of this trap. The outside is *covered* with 2 x 2 inch mesh and two concentric mesh cones pointing upstream funnel fish into the upper end of the trap. To deploy this gear, it is rolled down the bank with the open end downstream and-placed in a deep (at least eight feet) run along a bank. To fish the gear, it is simply rolled up the bank via cables and a winch until it is partially dewatered. Doors on the side of the cod end provide access for removing the catch with a dip net.

Gear efficiency is a function of channel configuration (depth, width), discharge, and turbidity. A single unit of gear in a large river is very inefficient so a number of units are required to capture a sufficient portion of a run. For example, in the Chehalis River, where we have fished this gear in each of the last five years, we employ seven hoop traps and catch 2-3% of the coho run (500-1,000 fish).

FIELD TRIAL

We placed one hoop trap into the lower Yakima River at river mile 2.0 off the right bank on October 1 at 2000 hours. This trap fished continuously until it was removed from the river at 0730 hours on October 8. We checked the trap and removed the captured fish each morning and evening. Over these seven days we captured only seven salmonids: 2 jack chinook, 2 male steelhead (1 wild, 1 hatchery), 1 adult male coho and 2 jack coho.

Very little activity was observed that would indicate any salmon were passing this point during these seven days so we were not surprised that so few fish were caught. Based on the numbers and timing of chinook passing Prosser, however, it appsars that some fall chinook may have been sntering the lower river during the first week of October.

The first fall chinook generally arrive at Prosser in late-August and early-September, so entryinto the lower river probably occurs at least a wssk or two earlier. High water temperatures are a concern, particularly early in the run, but also extending through September. For this reason, it may not be practical to capture, handle and tag fish in the lower Yakima River. Water temperatures during this first week of October ranged from 58° to 63°F.

The basic design of an adult enumeration effort would involve capturing enough fish in the lower river over the entire run so that a portion of the run could be tagged. Fish passing Prosser Dam could then be sampled for tags and an estimate of total spawners made. High water temperatures aside, another potential problem with trapping so low in the river is the likely catch and tagging of strays that fail to migrate upstream. This has the same effect as losing tags and results in biasing estimates high. This problem could be avoided or greatly reduced by trapping fish further upstream.

Consideration of trapping adults, on a trial basis, for enumeration purposes may best be accomplished at the fishways at Horn Rapids Dam. This would also solve other logistical problems associated with placing hoop traps in the lower river; limited access, navigation blockage, vandalism, etc.

DOWNSTREAM MIGRANT TRAPPING IN 1991 AT ROSA DAM

YKFP evaluation plans include comparing performance of hatchery produced spring chinook to that of wild spring chinook. These plans require capturing wild chinook smolts in sufficient numbers in the upper Yakima River and in the Naches River. The capability to capture the requisite numbers doss not currently exist upstream of the confluence of these rivers. In addition, it is uncertain how many wild spring chinook smolts remain upstream of this confluence until Spring. Without this knowledge, trapping facility designs and field efforts required to fulfill objectives prescribed in the experimental design plans are difficult or impossible to develop..

In cooperation with the YIN, we began to work on answering these questions in Spring 1991, installing a floating downstream migrant trap below Rosa Dam. Concurrently, the YIN continuously operated a trap in the Rosa Dam smolt bypass. Objectives of this combined effort include:

1. Estimating the number of spring chinook smolts remaining upstream of this point after mid March;

2. Determining the capture efficiency of the mobile gear and the smolt bypass trap on spring chinook, hatchery steelhead and hatchery sockeye smolts;

3. Determining migration timing for the various species past this point;

4. Assessing feasibility and resultant costs of these operations; and

5. Providing emigration information on hatchery steelhead released as part of the interaction work underway in the upper Yakima River.

On March 19, we installed a floating screw trap below Rosa Dam. This is the same trap used in the lower Yakima River in 1990 (For a description of this gear see 1990 TRAPPING GEAR AND OPERATION section of this report). We replaced the screw assembly of this trap with one that had stronger flights and a steeper pitch to avoid the problems encountered in 1990 of breaking the flights. Assembly was easily accomplished in one day with the assistance of a Bureau of Reclamation 25 ton crane.

We placed the trap approximately 100 yards downstream of the dam about 10 yards off the left bank. We began fishing on the evening of March 19 and stopped for the season on May 29. The gear was fished continuously throughout this period, requiring virtually no attention from the two man crew other than to remove and process the catch each morning and evening. This fishing schedule permits separating 24 hour catches into day and night components.

Over the season, we captured the following numbers of salmonids:

spring chinook	1,040
steelhead	497
sockeye	706
coho	18

To estimate capture efficiency of both trapping systems, several groups of branded wild spring chinook smolts, hatchery steelhead smolts and hatchery coho smolts were released. In addition, various groups of branded sockeye smolts were released in the upper Yakima River around Cle Elum. Initial indications are that efficiency of the screw trap was around 2-3%. These data will require more analysis to assess species specifia rates and the influence of discharge on efficiency. However, if capture efficiency is around this rate, then the pre-season project da of over 100,000 spring chinook smolts remaining above Rosa Dam appears to be an overestimate.

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